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PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF AIR POLLUTANTS ON SOYBEAN PLANTS

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake and stream systems, and the development of predictive models on the movement of pollutants in the biosphere..

This report describes a study to determine the effects of NO_2 and SO_2 on soybeans.

James C. McCarty
Acting Director

ABSTRACT

The objective of this study was to determine the effects of NO_2 and SO_2 on soybeans which may cause irreversible damage. The effects of NO_2 and SO_2 on total protein, RNA, DNA and protein synthesis were determined. Also, the effects of NO_2 on amylase, acid phosphatase, peroxidase and lipase were studied.

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INTRODUCTION

The project on "Physiological and Biochemical Effects of Air Pollutants on Soybean Plants" was initiated in 1974. The report on the investigation results involves several parameters, and therefore will be dealt with categorically on a yearly basis and on specific sections of studies. Interim reports described several important findings on the toxic effects of nitrogen dioxide (NO_2) on moisture content and morphological changes of selected plants. This final report is a comprehensive description of the over-all physical and biochemical effects on the plant, emphasizing the major changes that nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) produced on the cellular level.

The original proposal was designed to investigate the effects of NO_2 and SO_2 on both soybean and cotton plants. During the first year of the project it became apparent that the initial scope of work was too broad for the available resources. Thus, due to time constraints and personnel limitations, the study was restructured and confined to soybean plants only.

OBJECTIVES

The objective of this study was to investigate the effects of NO_2 and SO_2 on soybeans which may cause irreversible damage and a resultant decrease in crop production. The project was divided into three phases:

I. (First Year) Establishment of proper lethal and sublethal dosages of NO_2 and SO_2 to which the plants would be exposed. This involved determination of the exposure period as well as the quantity of these pollutants. The first month of the project period was devoted to acquiring personnel and proper facilities, including the fumigation chamber, refrigerated centrifuge and a spectrophotometer. The latter half of the year involved the calibration of the desired experimental dosages of the gases and the establishment of adequate greenhouse conditions for the test plants.

II. (Second Year) Research during this period concentrated on three areas:

(a) Physiological and morphological changes occurring under NO_2 exposure, with 0.2, 0.5, and 0.8 ppm concentrations at 2-hr, 4-hr, 6-hr, and 8-hr periods.

(b) Effects of NO_2 on total protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) contents of the leaf tissue. (The results of

this study were substantiated by the findings on RNase determination of the leaf tissue under identical experimental conditions.)

(c) Effects of NO_2 on the four selected enzymes, α-amylase, acid phosphatase, peroxidase and lipase, that are vitally important for plant survival and growth.

III. (Third Year) The findings during the second year resulted in an extension of the investigation to a study of the effects of SO_2 exposure under the same experimental conditions. This phase concentrated on:

(a) Effect of various dosages of SO_2 on RNA, DNA, and total and soluble protein of the leaf tissue.

(b) Effect of NO_2 on the protein synthesis mechanism of the leaf tissue (C^{14}-leucine incorporation).

(c) Effect of NO_2 and SO_2 on the yield of soybean crops after the 62-day period.

OBSERVATIONS

The observations and findings are divided into three broad sections:

(a) Morphological changes that occurred due to NO_2 and SO_2 exposures.

(b) Biochemical changes that led to changes in protein content of leaf tissue.

(c) Alteration of a vitally important enzyme or enzymes in addition to changes in the C^{14}-leucine incorporation system of plant tissue (protein synthesis mechanism).

MATERIALS AND METHODS

Soybean seeds (Glycine max L., cv. Bragg) were sown in sterilized sandy loam soil in 11-cm clay pots. The pots were placed in a fiberglass greenhouse with average day and night temperatures of 30 C and 25 C, respectively. The average relative humidity during the growing period ranged from 40 to 60 percent, and the natural day-length and light intensity in the greenhouse were not supplemented.

Following emergence, the seedlings were thinned to 2 per pot. When the seedlings produced their first pair of true leaves, each pot was supplied with 80 ml of a one-half strength Hoagland and Arnon's nutrient solution. The plants were watered with tap water when needed. Seedlings were exposed to pollutant gas after 3 weeks from date of sowing.

Twenty pots containing 2 plants per pot were used for each exposure period. The pots were watered thoroughly before exposure. The fumigation chamber used in this study was a Scientific System's Twin-cubator. Sixteen pots were placed inside the chamber, and 4 pots were left outside to serve as a control (0 hr). A series of chamber and laboratory controls were used for each concentration and NO_2 and SO_2 treatment. The protein, RNA and DNA content of chamber and laboratory control plants were the same. The enzyme activities and C^{14}-leucine incorporation to the leaf tissue were also the same. Thus the data for control in this report were the average of both chamber and laboratory controls.

No additional CO_2 was added during NO_2 and SO_2 treatments. However, both the gases were diluted with air for proper concentration during the exposure period in the chamber. The concentration of NO_2 gas inside the chamber at any one time was either 0.2, 0.5, or 0.8 ppm. The concentration of NO_2 within the chamber was monitored continuously by a Dynascience Multipollutant Gas Analyzer, Model 410.

The plants were grown under greenhouse conditions (fiberglass) where average daily temperature of 29 C and a relative humidity of 40% was maintained. These same conditions were simulated when the plants were placed in the exposure chamber. Also, the plants were saturated with water before exposure to concentration levels of 0.2, 0.5, and 0.8 ppm.

Batches of 4 pots were removed at random from the chamber following 2, 4, 6, and 8 hours of exposure. The plants were left on the laboratory bench for a 24-hour recovery period under light. Plants were examined carefully for any visual signs of damage. The leaves were then harvested, placed in freezer bags and stored in a freezer for future analyses.

SAMPLE EXTRACTION FOR NUCLEIC ACID
AND PROTEIN DETERMINATIONS

The removal of chlorophyll and lipid from the leaf tissue and the grinding of the chlorophyll-free tissue were carried out with methanol following essentially the Schmidt and Thannhauser (1) procedure. The extraction of free nucleotides, RNA and DNA was made with perchloric acid (PCA) using the procedure of Ogur and Rosen (2). Two grams (fresh weight) of leaf tissue were extracted with cold absolute methanol (20 ml) by grinding in a chilled mortar and pestle. Some purified sand was added to the mortar to facilitate grinding. The homogenate was centrifuged in the cold for 15 minutes at 20,000 Xg (International Centrifuge, Model B-20A). The residue was washed with more methanol and the supernatants were discarded. The residue was then extracted with 10 ml of cold (10 percent) PCA, and centrifuged for 15 minutes. The supernatant was refrigerated for future determination of free nucleotides. The residue was washed twice with 10% cold PCA (5 ml each time), and the solution was discarded. Twenty ml of 10% PCA was added to the residue and allowed to stand in a refrigerator for 24 hours before centrifugation. The supernatant was decanted and refrigerated for future RNA analysis.The residue was washed twice with 5 ml each of 10% cold PCA, centrifuged, and the solution was discarded. Ten ml of 10% PCA was added to the residue and heated for 15 minutes in a water bath maintained at 90 C. The samples were stirred a few times while heating, then cooled at room temperature and centrifuged. The supernatant containing DNA was stored in cold until analysed.

The residue was washed twice with 10% PCA and the washing was discarded. Protein was then extracted from the residue by adding 4 ml of 1 N NaOH followed by thorough stirring. This was allowed to stand overnight at room temperature. The tube content was transferred to a measuring cylinder and the volume adjusted to 50 ml with distilled water. The solution was allowed to stand for 2 hours and an aliquot of the clear supernatant was used for protein determination.

For the extraction of soluble protein, freshly harvested leaves (weighed) were homogenized in 0.1M phosphate buffer, (pH 7.0) in cold, and centrifuged. The supernatant was collected. The residue was washed twice with 5 ml of the same buffer and centrifuged. The collective supernatant was stored in a freezer with the original supernatant. The combined supernatant designated as soluble protein was precipitated with trichloroacetic acid (TCA) and processed exactly the same way as was done with the total protein extraction.

ANALYTICAL PROCEDURES

Protein Determination

Protein content was determined following Lowry's folin-phenol reagents (3) which are as follows:

Solution A. Dissolve 20 grams of Na_2CO_3 in distilled water and increase the volume to 500 ml. Dissolve 4 grams NaOH in 500 ml of water and mix together.

Solution B_1. Dissolve 2 grams of sodium potassium tartrate in 100 ml of water.

Solution B_2. Dissolve 1 gram of $CuSO_4$ in 100 ml of water.

Solution C. Mix: a. 50 ml of solution A
b. 0.5 ml of solution B_1
c. 0.5 ml of solution B_2

Procedure

To 1 ml of sample or blank, 5 ml of solution C was added and mixed thoroughly. After 10 minutes, 0.5 ml of diluted phenol reagent (5 ml of reagent plus 5 ml of water) was added and mixed in a vortex mixture. The mixture was allowed to stand for 30 minutes and the absorbance was recorded in a Bausch and Lomb Spectronic-20 at 500 mμ. An albumin standard was run with each batch of sample determinations. Protein was calculated per gram of wet or dry tissue.

Nuclic Acid Determination

RNA was determined following Ogur and Rosen's method (2) using ribose determination with orcinol ferric chloride reaction. Highly polymerized yeast RNA was used as a standard. The blank contained 1 ml of 0.5 N PCA, 2 ml of $FeCl_3$, 6 ml of water, and 0.1 ml of orcinol reagent, while in the sample tubes PCA was replaced by the same volume of the sample. After thorough mixing, a marble was placed on the test tube mouth and the tube was heated in a boiling water bath for 15 minutes. After cooling at room temperature, the optical density of the green chromogen was read at 627 mμ in a colorimeter.

DNA was determined by the diphenylamine reaction as described by Dische (4) with slight modifications. Two ml of diphenylamine reagent was used instead of 4 ml and the sample was placed in a boiling water bath for 15 minutes instead of keeping it overnight at room temperature. The tubes were read within 30 minutes of the color development. Calf thymus DNA was used as a standard.

Enzyme Assays

From the study of the stability of enzymes it was noted that the enzymes concerned in this investigation were fairly stable when left extracted in a frozen condition. The extracted supernatant was left up to 48 hours in the freezer and batches of samples were assayed to eliminate inconsistencies. In a separate experiment the enzyme activities from fresh leaves were compared with those from frozen samples and were found to be closely related. For

subsequent experiments, frozen samples from experimental plants were used.

One gram of frozen leaf tissue was found in a chilled mortar and pestle with 25 ml of phosphate buffer (pH 7.2) for about 10 minutes. Some purified sand was added to facilitate grinding. The homogenate thus obtained was spun in a refrigerated centrifuge at 20,000 Xg for 15 minutes. The supernatant was decanted and used for enzyme assay. Activities of various enzymes were measured according to the following procedures: alpha-amylase (5), acid phosphatase (6), peroxidase (7), and lipase (8).

L-leucine-C^{14} Incorporation to Leaf Homogenate

The incorporation method was a modification of a procedure used by Marcus et al. (9). Immediately after collection of the trifoliate leaves, preparations were made for incorporation. One-gram samples from each exposure time (½, 1, 2, 4, 6, and 8 hours) and control were weighed and placed in individual mortars. The samples were then homogenized in cold (in an ice bath) with 5 ml of cold phosphate buffer, 0.2M, pH 6.0.

Twenty-four 50-ml Erlenmeyer flasks, including 3 blanks were labeled so that experimental samples were run in triplicate. The blanks received 1 ml of 0.2M phosphate buffer (pH 6.0), 1 ml of leaf homogenate from control samples, and 2 ml of 10% TCA. Each sample received 0.1 ml of 0.25 microcurie L-leucine-C^{14} followed by 0.5 ml of fresh guanosine triphosphate (GTP) (1 mg/ml), 0.4 ml of phosphate buffer (pH 6.0), and 1 ml of leaf homogenate from the appropriate hours of exposure and control. The samples were then incubated in a water bath for 1 hour.

After the 1-hour incubation period, 2 ml of 10% TCA were added to each sample to terminate the enzyme reaction. The denatured mixture was then transferred to centrifuge tubes with the aid of a washing media. The washing media contained 100 mg of L-leucine-C^{14} in 5% TCA. All samples were washed three times to remove the artificial radioactivity on the precipitate. After each wash the supernatant was discarded; after discarding the third wash, the precipitates were transferred to scintillation vials for drying. The samples were air dried and then 20 ml of scintillation liquid were added to each vial and counted in a liquid scintillation spectrometer. The protein content of the leaf homogenate was estimated by folin-phenol reagent following the method of Lowry et al. (3).

Since quenching of the precipitate may interfere with the actual protein synthesis, the precipitates were dried by various methods and the radioactivity of the experimental samples corrected. Samples dried with ethyl alcohol and ether did not give measurable counts. This is because ethyl alcohol removed the soluble protein in addition to the newly synthesized protein. The samples which were oven dried became charred and gave background counts. The CO_2 probably had been removed from the proteins during intensive heating. The precipitate was dried at room temperature and counted. The quenching did not affect the experimental results.

SO_2 Gas Fumigation

The fumigation chamber provides the facility to fumigate SO_2. The gas cylinder was connected to the chamber. Prior to SO_2 fumigation, the chamber was aired for 48 hours at a particular concentration of SO_2 and constantly monitored by the Dynascience Multi-pollutant gas analyzer. The concentration and the duration of exposure were the same as was used with NO_2 gas.

Recovery Experiment

All trifoliate leaves from the controls and treated plants were collected and stored in a freezer. Leaves with the same plastochron index (PI) values (see Appendix) were used for biochemical studies.

Batches of plants were returned to the greenhouse after defoliation for recovery experiments. For morphological changes and yield of crops, both NO_2- and SO_2-exposed plants were allowed to grow for 30 days and 60 days. For the $^{2}C^{14}$-leucine incorporation study, the defoliated plants were left at the greenhouse bench for an additional 23-day recovery period. During recovery, the plants were observed for abnormalities in growth, flowering, fruiting, and general appearance. At the end of the recovery period the morphological changes, yield of the seed pods, and number of seeds were noted. The other batch of samples (23-day recovery) were harvested for incorporation study.

Moisture Content

One gram of the control and exposed samples was oven dried at 110 C for 48 hours and the weights of the samples were estimated. The percent moisture content was determined from the differences of the weights before and after drying.

Plastochron Index (PI)

Plastochron indices were determined using procedures developed by Erickson and Michelini (10). These indices were used primarily for plant selection (especially on the basis of leaf development and maturity). The indexes were calculated from the following equation:

$$PI = n + \frac{\text{Log Ln} - \text{Log 20}}{\text{Log Ln} - \text{Ln} + 1}$$

where: PI = the Plastochron Index (the age of the plant expressed in plastochrons).

n = the serial number of the leaf, counting from the base of the plant, and including the leaf which is just longer than 20 mm or any other control length. The cotyledonary leaves are excluded from the measurement.

Log Ln = the logarithm of the length in mm of the leaf n which is just longer than 20 mm.

Log Ln + 1 = the logrithm of the length in mm of the leaf n + 1 which is just shorter than 20 mm or any other control length.

The leaf Plastochron Index is defined as the Plastochron Index minus the serial number of the leaf in question.

For example, if the Plastochron Index of the plant is 6.51 and the serial number of the leaf of interest is 4.00, the PI = 6.51 - 4.00, or 2.4 for the fourth leaf.

RESULTS

MORPHOLOGICAL CHANGES AND MOISTURE CONTENT OF NO_2 EXPOSED PLANTS

The soybean plants were watered thoroughly before placement in the fumigation chamber. Dry weights of the control (0 hour) plants ranged from 0.20 to 0.25 grams per gram of fresh leaf tissue (Table 1). In other words, the moisture content of the control leaves varied from 75 to 80%. Following exposure to 0.2 and 0.5 ppm of NO_2, the dry weights declined slightly, indicating that the leaves contained more moisture than the controls. This might be due to a decreased transpiration rate or increased water uptake, or both. Possibly the stomata were closed to prevent NO_2 absorption, and consequently, the water loss through these pores was diminished. Different results were, however, obtained when the plants were exposed to 0.8 ppm of NO_2. The dry weights of the treated leaf tissue were almost twice as much as those of the control plants (Table 1). The desiccation effects were clearly visible on the leaves of the treated plants. These leaves had papery-thin patches devoid of chlorophyll; the tissues in these areas appeared dry and dead. Because of such varied differences in the moisture content among the groups of treated plants, it would be erroneous to express and compare results on a fresh weight basis unless the moisture content of the tissue was also indicated.

The effect of SO_2 on the leaf followed the same trend as was observed with NO_2. The effect was extensive. The discoloration was more intensive. The appearance of yellow color on the leaf surface was scattered and less intensive than that with NO_2.

PROTEIN CONTENT

Upon exposure of the plants to 0.2 ppm of NO_2, the protein content of the leaves increased up to a period of 4 hours and then decreased slightly (Table 2). It appears that the plant tissue reacts to a small dosage of NO_2 in a manner similar to the response of any living tissue to a sub-lethal dosage of poison. The plant apparently can recognize the potential danger and tries to combat it by stimulating its metabolism as indicated by the increased synthesis of protein (Figure 1).

The protein content of the leaf tissue increased from 54 mg to 69 mg per gram of dry tissue, following exposure to NO_2 at 0.5 ppm for 2 hours (Table 2). Increasing periods of exposure caused a steady decline, and at 8 hours the protein content dropped to only 15 mg. At this relatively high dosage of pollutant, the plant initially tries to cope; however, with

TABLE 1. MOISTURE CONTENT OF SOYBEAN LEAF TISSUE AS AFFECTED BY NO_2 AND SO_2 GAS

Time of Exposure (hr) ppm	SO_2 Gas Percent Moisture*			NO_2 Gas Percent Moisture*		
	0.2	0.5	0.8	0.2	0.5	0.8
0 Lab	75.0	74.0	75.0	82.5	83.1	82.5
0 Chamb.	75.0	75.0	75.0	-	-	-
½	76.0	77.0	69.0	83.8	76.0	77.4
1	76.0	77.0	65.0	84.3	64.8	80.0
2	77.0	76.0	63.0	69.5	61.2	59.0
4	78.0	76.0	62.0	64.0	55.1	62.0
6	75.0	74.0	49.0	64.2	57.0	57.3
8	74.0	74.0	48.0	60.7	58.5	57.2

*Average of three individual experiments.

TABLE 2. EFFECT OF NO_2 GAS ON THE PROTEIN CONTENT OF SOYBEAN LEAF TISSUE

ppm NO_2 Gas	Mg Protein/gm Dry Tissue*				
	Control	Hours Exposure			
		2	4	6	8
0.2	42.5	89.0	83.5	65.0	72.0
0.5	50.6	72.0	60.6	23.0	32.5
0.8	48.4	12.5	10.8	8.6	8.6

*Average of 5 individual experiments (exposures).

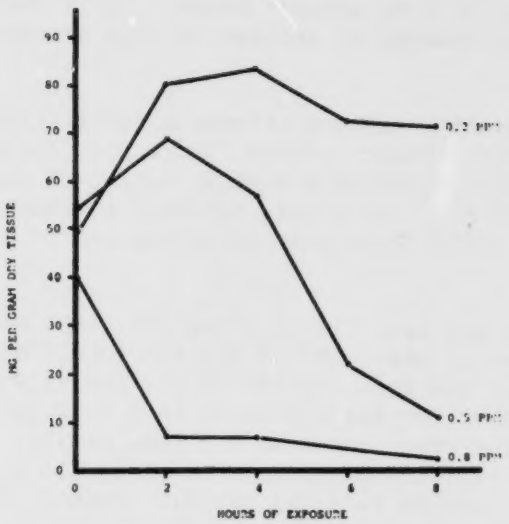

Figure 1. Effects of NO_2 exposure on protein content of soybean leaf tissue.

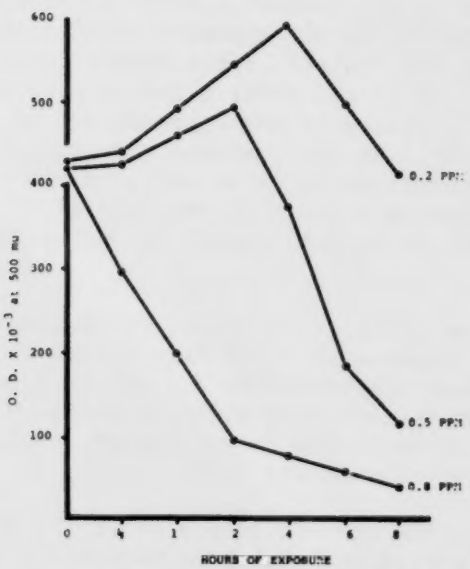

Figure 2. Total protein content of soybean leaf tissue as affected by SO_2 gas.

prolonged exposure, the NO_2 concentration inside the plant becomes too high for the plants to protect themselves. Apparently, the proteins in the plants undergo degradation and the machinery for new protein synthesis is also damaged.

Upon exposure of the plants to 0.8 ppm of NO_2, the protein content of the leaves dropped rapidly. From a control value of 40 mg of protein per gram of dry tissue, it dropped to only 7 mg following 2 hours of exposure, and to a negligible value of 3 mg after 8 hours. It is obvious that the plants would suffer severe damage if exposed to this concentration for even a short period.

These results on protein content offered a correlation between the concentrations of NO_2 and the exposure period (Table 2). The plants exposed to 0.2 ppm for 6 to 8 hours suffered a similar degree of damage as those exposed to 0.5 ppm for 2 hours. The plants suffered a greater degree of damage when exposed to 0.8 ppm for 2 hours than those exposed to 0.5 ppm of NO_2 for 8 hours.

Protein content of the leaf tissue after the plant was exposed to SO_2 followed the same pattern as was noted on NO_2 exposure (Table 3). At 0.2 ppm protein content increased and then started to decline at 4 hours of exposure. At 0.5 ppm, protein content increased only up to 1 to 2 hours of exposure and then declined. On the other hand, at 0.8 ppm, protein content started to decrease even at one-half of exposure. The change in soluble protein content was the same as that found in total protein content (Figure 2).

RNA CONTENT

Since RNA is involved in the protein synthesis mechanism, any change in the contents of total RNA due to NO_2 exposure would affect its over-all impact on the plant. Total RNA content of the leaves increased from 9.0 mg per gram of dry leaf tissue to 27 mg, after a 2-hour exposure to 0.2 ppm of NO_2 (Table 4). The 3-fold increase in RNA corresponded to the increased value of protein at this time. As RNA precedes protein synthesis, NO_2 presumably stimulated RNA synthesis which is so vital to plant survival in a polluted environment. Following 4 hours of exposure at this concentration, the RNA content dropped back to control level; it decreased further with increasing exposure periods.

With 0.5 ppm, the same stimulatory effect was evident after a short exposure period. The 67% increase as noted here is, of course, quite low as compared to the 300% increase observed with 0.2 ppm of NO_2. This increase level of RNA was maintained throughout the entire exposure period. In spite of the high levels of RNA noted in the 6- and 8-hour samples, the protein content of the corresponding samples was quite low.

Upon exposure to SO_2, RNA content increased at the 2-hour period with 0.2 ppm and at the 1-hour period with 0.5 ppm, maximally, up to 20 mg/per gram of leaf tissue (Table 5). There was a decline in RNA content at the

TABLE 3. PROTEIN CONTENT OF SOYBEAN LEAF TISSUE AS AFFECTED BY SO_2 GAS

	mg/gm Wet Tissue*								
	Total			Soluble			Insoluble		
	0.2	0.5	0.8	0.2	0.5	0.8	0.2	0.5	0.8
0 Lab.	17.0	17.2	17.7	15.0	14.7	14.9	1.3	1.6	1.5
Cham.	16.5	17.2	16.5	15.3	14.9	14.6	1.5	1.5	1.4
½	17.5	17.2	12.8	15.8	15.9	9.8	1.9	1.7	1.4
1	19.4	18.8	8.7	17.8	16.3	7.0	1.9	1.9	1.0
2	22.0	19.9	3.8	19.8	18.2	2.8	2.1	1.9	0.9
4	23.9	15.5	3.0	22.0	13.9	1.9	1.9	1.9	0.8
6	20.2	7.3	2.3	18.5	6.2	1.4	1.7	1.1	0.8
8	17.1	4.9	1.6	16.5	3.4	1.7	1.6	0.9	0.5

*Average of 3 individual experiments.

TABLE 4. RNA AND DNA CONTENTS OF SOYBEAN LEAF AS AFFECTED BY NO_2 GAS

Hours Exposure	mg/g Dry Tissue*		
	0.2 ppm	0.5 ppm	0.8 ppm
RNA			
0	9.0	7.6	10.5
2	27.0	12.7	7.0
4	9.0	9.0	6.5
6	4.5	14.0	7.0
8	7.0	12.0	9.0
DNA			
0	2.7	2.8	4.5
2	2.9	4.0	3.9
4	2.3	4.4	3.4
6	1.4	2.6	2.7
8	2.3	3.0	4.0

*Average of 4 individual exposures.

TABLE 5. RNA AND DNA CONTENTS OF SOYBEAN LEAF AS AFFECTED BY SO_2 GAS

Hours Exposure	mg/g Dry Tissue*		
	0.2 ppm	0.5 ppm	0.8 ppm
RNA			
0	14.6	8.2	9.3
½	9.1	12.2	12.5
1	10.4	20.2	11.6
2	20.0	6.6	10.8
4	14.6	12.6	10.0
6	14.6	7.3	10.0
8	11.4	6.4	10.8
DNA			
0	3.2	2.5	2.9
½	2.9	3.6	3.5
1	2.6	2.4	3.0
2	2.1	2.5	2.6
4	2.6	2.9	2.4
6	1.9	3.0	2.8
8	2.9	2.7	2.9

*Average of 4 individual exposures.

6- to 8-hour exposure period with 0.5 ppm SO_2.

DNA CONTENT

The DNA content of the untreated soybean leaves ranged from 2.7 to 4.5 mg per gram of dry weight (Table 4). Following exposure to NO_2, no appreciable changes in the DNA content were observed.

DNA content of the leaves exposed to SO_2 remained unchanged at various periods of exposure, even with higher dosages.

ENZYME ACTIVITIES

α-Amylase activity gradually decreased at 8 hours of exposure. Maximum decrease was found with 0.8 ppm NO_2 (Figure 3). Acid phosphatase activity also decreased at 8 hours with 0.8 ppm NO_2 (Table 6). The decrease was not as marked as that noted with α-amylase. In contrast to the activities of these two enzymes, peroxidase activity slightly increased (8-9%) (Table 7). Lipase activity, however, increased by 66% at 8 hours with 0.8 ppm NO_2 (Figure 4). It is evident that the effect of NO_2 on α-amylase, acid phosphatase and lipase is prominent, while 0.8 ppm NO_2 at 8 hours was ineffective on peroxidase.

PROTEIN SYNTHESIS (C^{14}-LEUCINE INCORPORATION TO PROTEIN)

The results of C^{14}-leucine incorporation in the leaf tissue of the fumigated plants indicated that there was no alteration of the protein synthesis mechanism at all dosages and exposure periods. NO_2 did not inhibit protein synthesis (Figures 5 and 6).

Contrary to this result, it was noted that SO_2 affected the protein synthesis mechanism dramatically. At all levels of SO_2, C^{14}-leucine incorporation per gram of leaf tissue decreased more than 70%. It appears, therefore, that SO_2 is not only toxic to the plant leaf cell but also to the protein synthesis (Figure 6).

EFFECT OF AIR POLLUTANTS ON YIELD OF CROPS

The yield of seed pods after the 62-day recovery period, after exposure to NO_2 gas, was significant (Table 8). For each exposure concentration the controls produced 8 to 9 seed pods. With 0.2 ppm NO_2 gas, there was a 23.5% drop in the number of seed pods per plant (up to 1-hour exposure time). A further decrease of 49% was noted at 4 hours. The number of seed pods per plant then started to increase rapidly. At 6 hours of exposure, the number of seed pods for recovery plants was 30% less than the controls. At 8 hours, the number of seed pods per plant reached its control value. In spite of the toxicity of NO_2 gas in the protein synthesis machinery, the yield of seed pods per plant remained the same as that of the control plants. This result is striking, because with 0.2 ppm NO_2 gas at shorter exposure times, toxicity of the pollutant affected the yield. At prolonged exposure times, plants produced an increased number of seed pods per plant. With

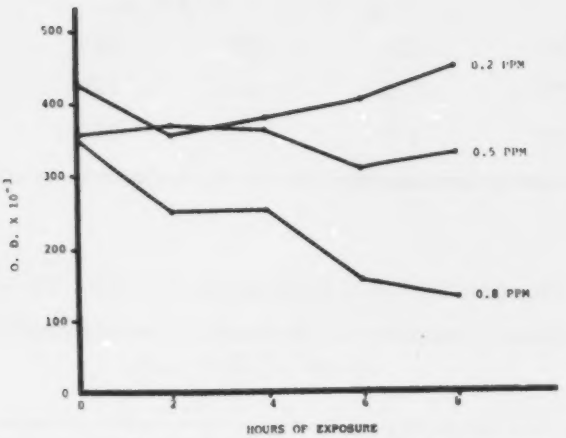

Figure 3. Changes in alpha-amylase activity in soybean leaf tissue after exposure to NO_2 gas.

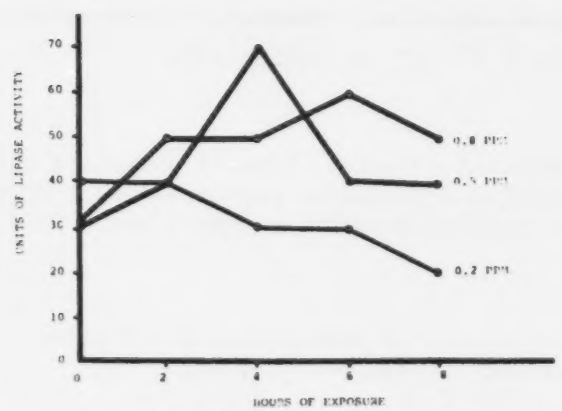

Figure 4. Changes in lipase activity in soybean leaf tissue following NO_2 exposure.

TABLE 6. ACID PHOSPHATASE ACTIVITY OF SOYBEAN LEAF TISSUE AS AFFECTED BY NO_2 GAS

Conc. of NO_2 (ppm)	Hours of Exposure				
	0	2	4	6	8
	O.D. X 10^{-3} at 405 mμ				
0.2	232	227	230	265	266
0.5	185	125	130	125	130
0.8	230	230	225	200	192

TABLE 7. EFFECT OF NO_2 GAS ON THE PEROXIDASE ACTIVITY IN SOYBEAN LEAVES

Conc. of NO_2 (ppm)	Hours of Exposure				
	0	2	4	6	8
	O.D. X 10^{-3} at 420 mμ				
0.2	510	450	420	460	470
0.5	400	450	420	460	450
0.8	350	405	390	370	380

O.D. was obtained after 5 min of incubation time.

Reaction Mixture

One ml of 0.5% Catechol in water, 6 ml of phosphate buffer (0.05 M, pH 6.1), 0.5 ml of purified horseradish peroxidase 61.5 PZ units/mg or 100 μg/ml in phosphate buffer; incubation time 5 min. Total vol. 7.5 ml.

Enzyme + Peroxidase

One ml of Catechol (conc. as above), 6.0 ml of buffer, 0.5 ml of peroxidase or leaf extract. Total vol. 7.5 ml.

Blank

6.5 ml buffer, 1 ml water. Total vol. 7.5 ml.

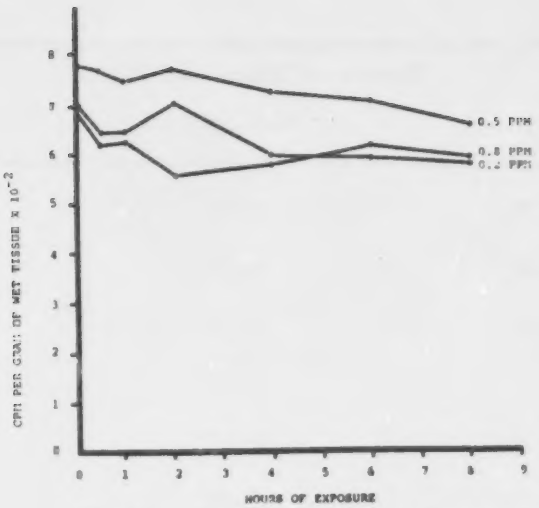

Figure 5. L-leucine C^{14} incorporation to soybean leaf protein as affected by NO_2 fumigation.

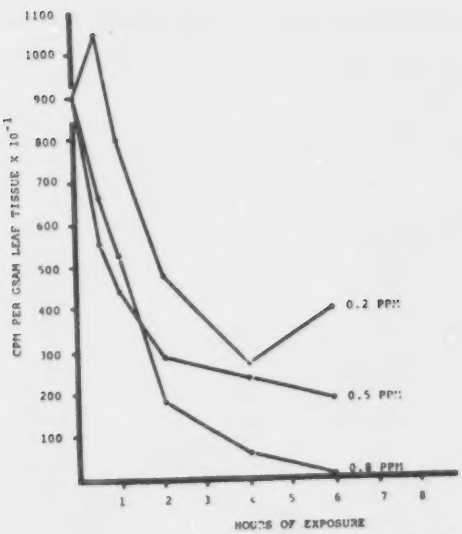

Figure 6. L-leucine C^{14} incorporation to soybean leaf protein as affected by SO_2 fumigation.

TABLE 8. YIELD FROM PLANTS GIVEN NO_2 GAS EXPOSURES OF 0.2, 0.5, AND 0.8 ppm

Time in Hours	Number of Pods Per Plant		
	0.2 ppm	0.5 ppm	0.8 ppm
0	8.25	8.50	9.0
½	6.50	4.50	8.5
1	6.50	5.00	3.75
2	5.0	4.75	4.0
4	4.25	8.00	4.25
6	5.75	8.00	6.25
8	8.75	8.75	8.50

0.5 ppm NO_2 gas, there was a sharp drop in the yield up to 2 hours of exposure. The decrease was 41 to 42%; thereafter, the yield rose to 66 and 67% at 4 and 6 hours exposure, respectively. There was a further rise at 8 hours of exposure. This rise brought the yield back to its control values. It appeared that at this level of NO_2 gas pollution for shorter exposure times, plants responded to toxicity of the gas. In general, growth was retarded and the number of seed pods per plant was diminished. At extended exposure times seed pod production increased. The plants not only overcame the toxicity, they showed increased yield. The results from 0.8 ppm were similar to those obtained with 0.5 ppm, NO_2 gas. The control values were 9 seed pods per plant and the number of seed pods per plant started declining at the very start of exposure. At 1 hour of exposure, the number of seed pods per plant declined to 3.6 seed pods per plant (a decrease of 60% over the control). It remained at this low level to 4 hours of exposure, then sharply increased to 8.5 seed pods per plant at 8 hours of exposure. The recovery, therefore, increased to 36% from the 4-hour exposure period to the 8-hour exposure period. The yield of the plants recovering from 0.2, 0.5 and 0.8 ppm NO_2 gas, at ½ hour to 8 hours of exposure revealed that the yield did not exceed 8 to 9 seed pods per plant (the value of the controls). Nitrogen dioxide at low levels produced toxicity to plant yield (Figures 7 and 8).

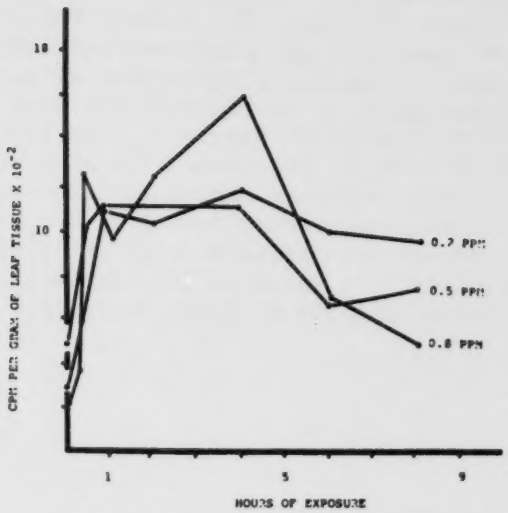

Figure 7. L-leucine C^{14} incorporation to soybean leaf protein as affected by NO_2 fumigation (23 days recovery).

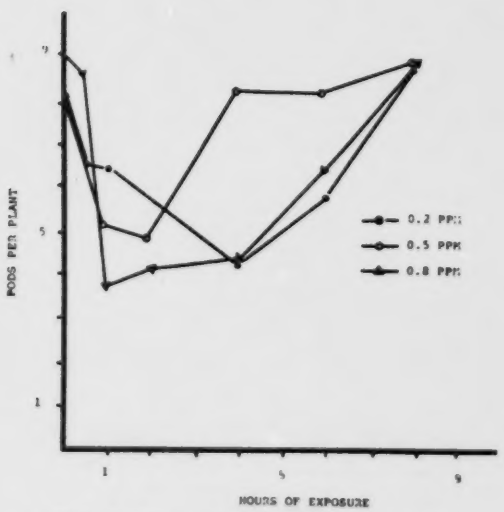

Figure 8. Total yield of seed pod from soybean plants fumigated with NO_2 (62 days recovery).

DISCUSSION

This report is the result of the study on the effects of NO_2 and SO_2 air pollutants on soybean plant tissue completed during 1975-1978. The investigators studied physiological and biochemical changes in soybean plants as affected by NO_2 and SO_2 fumigation.

Earlier results indicated that NO_2 gas affected the concentrations of protein and RNA in soybean leaves. At three exposure concentrations (0.2, 0.5 and 0.8 ppm) with various times of exposure (½ hr to 8 hrs), both RNA and protein contents of leaf tissue initially increased and then decreased with higher dosages of the gas. The effects with high concentrations of the gas and extended periods of exposure were irreversible. However, there was no change in DNA content.

Extending the previous investigation on the basis of the original proposal, the plants were exposed to SO_2 gas in a manner described previously in this report, and protein, RNA and DNA were determined. The results are divided into two distinct parts:

I. The changes in protein, RNA and DNA contents of soybean leaf tissue as affected by SO_2 fumigation.

The alteration of C^{14}-leucine incorporation to the leaf protein (*in vitro* study) as affected by SO_2 and NO_2 fumigation. It is evident that protein content of the SO_2-exposed leaf tissue was changed, increasing with low levels and short periods of exposure and decreasing with high levels and extended periods of exposure. While protein content changes caused by SO_2 gas did not follow the pattern produced by NO_2 gas, there is a close relationship between the two results. The increase in RNA content appeared to follow the increase in protein content. DNA content of the leaf tissue exposed to SO_2 gas remained at a constant level, which is similar to the result obtained on exposure to NO_2 gas.

II. The second part of this investigation involved incorporation of C^{14}-leucine to leaf protein and the effects of NO_2 and SO_2 fumigation on protein synthesis.

The recovery of the plants (23 days and 62 days) showed higher yield on NO_2 exposure. NO_2 had little or no effect on C^{14}-leucine incorporation to leaf protein, indicating the ineffectiveness of NO_2 to protein synthesis. The findings, therefore, suggest that changes in protein, RNA contents and that in the α-amylase, peroxidase and lipase activities were not due to the alteration of the rate of protein synthesis. In contrast to this result,

there was a sharp decrease in the amino acid incorporation to protein by SO_2 gas. The initial rise at 0.2 ppm up to 1 hour exposure was reproducible. The decline in incorporation at higher dosages of SO_2 indicated that SO_2 is toxic to the protein synthesis mechanism. It may also be possible that SO_2 upon entering the cell, forms a complex with the cellular components which, in fact, is inhibitory to the enzyme or enzyme system involved in incorporation of C^{14}- leucine to protein.

It is not known if the accumulated gases inside the cell change the pH of the cellular material. Any acidity (through the dissolution of NO_2 and SO_2 gases) in the cell might change the optimum activity of the enzymes concerned with C^{14}-leucine incorporation, which must be maintained throughout the plant's life. It is presumed that the prolonged entry of NO_2 or SO_2 gas through stomata exerts an osmotic shock on the particulates of leaf cells, resulting in the release of a number of enzymes that are involved in lytic metabolism.

In the recovery experiments, plants exposed to NO_2 gas not only survived, but also showed stimulated growth. The yield of seed pods and the number of seeds increased, compared to those of the control. The findings, therefore, indicated that NO_2 gas (at concentrations used in this study) was beneficial to the yield to crops. From the C^{14}-leucine incorporation (preliminary experiments) it is apparent that the recovery study needs further experimentation for confirmation.

This study shows that both NO_2 and SO_2 react differently at different concentrations. Both RNA and protein contents were increased at lower concentrations of NO_2, whereas at higher concentrations of NO_2 and SO_2 both protein and RNA were decreased (Tables 4 and 5, Figures 3 and 4). Toxicity of both of these gases was visible at 0.5 ppm during 4 hrs exposure period. The beneficial effects of NO_2 in plants as a whole are not surprising, since it is known that nitrate and thiourea induce the nitrate reductase activity (12). The increased crop yield in the recovery experiment may be due to the production of nutrients from NO_2 by enzymatic reduction. Recent work of Zeevaart (11) on NO_2 fumigation of pea plants resulted in increased ammonia formation. It appears that NO_2 gas increased the nitrate reductase activity in pea plant tissue (12). This indicates that NO_2 acts as a potential substrate for the enzyme (nitrate reductase) already known to be present in the leaf tissue. The enhancement of the substrate may or may not increase the turnover rate of the enzyme, nitrate reductase. Since both soybeans (used in this study) and peas are leguminous plants, the increased crop yield in NO_2 gas fumigated plants might have a similar effect as in the peas in Zeevaart's experiment (11).

The increased protein content in NO_2-fumigated plants did not follow the C^{14}-leucine incorporation to the plant leaf tissue. It should be noted that C^{14}-leucine incorporation is the index of rate of the protein synthesis but not the increase of the turnover rates of the enzymes involved in protein synthesis.

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APPENDIX

A. NO_2 EXPOSURE

B. SO_2 EXPOSURE

APPENDIX A

NO_2 FERMIGATION

Tables

1. Soybean leaf plastochron indexes - NO_2 0.2 ppm
2. Soybean leaf plastochron indexes - NO_2 0.5 ppm
3. Soybean leaf plastochron indexes - NO_2 0.8 ppm
4. Moisture contents of conbial and NO_2 exposed plants
5. Data from the first exposure to NO_2 to soybean plants at 0.2, 0.5, and 0.8 ppm
6. Data from the second exposure to NO_2 to soybean plants at 0.2, 0.5, and 0.8 ppm
7. Data from the third exposure to NO_2 to soybean plants at 0.2, 0.5, and 0.8 ppm
8. Data from plants recovered from NO_2 gas fermigation at 0.2, 0.5, and 0.8 ppm

Figures

1. a,b,c; L-Leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.2, 0.5, and 0.8 ppm (exposure 1)
2. a,b,c: L-Leucine C^{14} incorporation to soybean leaf tissue to NO_2 at 0.2, 0.5, and 0.8 ppm (exposure 2)
3. a,b; L-Leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.2 and 0.5 ppm (exposure 3)
4. A combined graph of all incorporation of L-Leucine C^{14} to soybean leaf tissue at 0.2, 0.5, and 0.8 ppm
5. a,b,c; L-Leucine C^{14} incorporation to soybean leaf tissue from plants recovered from 0.2, 0.5, 0.8 NO_2 exposure
6. a,b,c; yield of plants after 62-day recovery from 0.2, 0.5, and 0.8 ppm NO_2 exposure

TABLE 1

SOYBEAN LEAF PLASTOCHRON INDEXES
NO_2-0.2 ppm

Time (hrs)	Mean PI Values			Mean LPI Values		
	Exposure I	Exposure II	Exposure III	Exposure I	Exposure II	Exposure III
0	1.43	2.02	2.12	0.43 ± 0.60	1.01 ± 0.024	1.1 ± 0.12
½	1.53	2.10	2.08	0.53 ± 0.56	1.10 ± 0.13	1.08 ± 0.07
1	1.6	2.16	2.10	0.6 ± 0.48	1.16 ± 0.10	1.10 ± 0.11
2	1.68	2.10	2.06	0.68 ± 0.57	1.10 ± 0.14	1.06 ± 0.08
4	1.68	2.03	2.08	0.68 ± 0.57	1.03 ± 0.03	1.08 ± 0.16
6	1.70	2.03	2.09	0.70 ± 0.58	1.03 ± 0.030	1.09 ± 0.12
8	2.04	2.16	2.08	1.40 ± 0.45	1.60 ± 0.08	1.08 ± 0.16

TABLE 2

SOYBEAN LEAF PLASTOCHRON INDEXES
NO_2-0.5 ppm

Time (hrs)	Mean PI Values			Mean LPI Values		
	Exposure I	Exposure II	Exposure III	Exposure I	Exposure II	Exposure III
0	2.10	1.73	1.89	1.10 ± 0.11	0.73 ± 0.62	0.89 ± 0.56
½	1.71	1.98	2.07	0.71 ± 0.59	0.98 ± 0.41	1.06 ± 0.07
1	1.79	1.41	1.95	0.79 ± 0.49	0.41 ± 0.57	0.95 ± 0.39
2	2.00	1.74	1.88	1.00 ± 0.41	0.74 ± 0.62	0.88 ± 0.35
4	1.79	1.94	1.84	0.79 ± 0.49	0.94 ± 0.39	0.84 ± 0.54
6	1.53	2.11	1.90	0.53 ± 0.57	1.11 ± 0.08	0.90 ± 0.36
8	2.01	1.99	2.04	1.01 ± 0.42	0.99 ± 0.41	1.04 ± 0.59

TABLE 3

SOYBEAN LEAF PLASTOCHRON INDEXES
NO_2-0.8 ppm

Time (hrs)	Mean PI Values			Mean LPI Values		
	Exposure I	Exposure II	Exposure III	Exposure I	Exposure II	Exposure III
0	2.11	2.14	2.14	1.11 ± 0.09	1.14 ± 0.1	1.22 ± 0.13
½	2.09	2.10	2.03	1.09 ± 0.07	1.10 ± 0.1	1.03 ± 0.06
1	2.02	2.05	2.21	1.02 ± 0.44	1.05 ± 0.08	1.21 ± 0.16
2	1.99	2.02	2.07	0.99 ± 0.41	1.02 ± 0.04	1.07 ± 0.07
4	1.69	2.01	2.07	0.69 ± 0.57	1.01 ± 0.03	1.07 ± 0.07
6	.07	2.05	1.79	1.07 ± 0.07	1.04 ± 0.04	0.79 ± 0.49
8	.10	2.85	1.92	1.10 ± 0.09	1.85 ± 0.53	0.92 ± 0.38

TABLE 4

MOISTURE CONTENTS OF CONTROL AND NO_2 EXPOSED PLANTS

Sample Exposure Time (hrs)	Sample Wet Weight (mg)	Mean Sample Dry Weight (mg)			Percent Moisture		
		0.2 ppm	0.5 ppm	0.8 ppm	0.2 ppm	0.5 ppm	0.8 ppm
Lab. Control (0 hrs)	1000	175.5	169	175	82.5	83.1	82.5
½	1000	162.5	240	226.5	83.75	76	77.35
1	1000	157.5	35.5	201.5	84.25	64.25	79.85
2	1000	305.5	388.5	412	69.45	61.15	58.8
4	1000	360	449	382	64	55.1	62
6	1000	358.5	430	427.5	64.15	57	57.25
8	1000	393.5	414	428	60.65	58.5	57.2

TABLE 5

DATA FROM THE FIRST EXPOSURE OF NO_2 GAS TO SOYBEAN PLANTS AT 0.2, 0.5, AND 0.8 ppm

Time (hrs)	CPM Per Gram Wet Tissue			Mean CPM Per Gram Wet Tissue		
	0.2 ppm	0.5 ppm	0.8 ppm	0.2 ppm	0.5 ppm	0.8 ppm
0	630.00 650.00	630.00 650.00	630.00 650.00	640 ± 14.14	640 ± 14.14	640 ± 14.14
½	353.75 343.75	705.00 745.00 861.00	597.50 585.00	348.75 ± 7.07	770.33 ± 81.03	591.25 ± 8.84
1	426.25 403.75	750.00 740.00 748.75	637.50 567.50	415 ± 15.90	746.25 ± 5.45	602.5 ± 49.50
2	192.50 177.50	777.50 841.25 701.25	662.50 771.25 578.75	185.00 ± 10.60	773.33 ± 70.10	670.83 ± 96.52
4	183.75 152.50	763.75 711.25 692.50	575.00 597.50 591.25	168.12 ± 22.10	722.50 ± 36.93	587.92 ± 11.61
6	531.25	716.25 652.50 748.75	560.00 716.25 633.75	531.25	705.83 ± 48.96	636.67 ± 78.17
8	422.50 406.25 341.25	692.50 620.00	658.75 713.75	390.00 ± 43.00	656.25 ± 51.26	686.25 ± 38.89

TABLE 6

DATA FROM THE SECOND EXPOSURE OF NO_2 GAS TO SOYBEAN PLANTS AT 0.2, 0.5, AND 0.8 ppm

Time (hrs)	CPM Per Gram Wet Tissue			Mean CPM Per Gram Wet Tissue		
	0.2 ppm	0.5 ppm	0.8 ppm	0.2 ppm	0.5 ppm	0.8 ppm
0	763.30 872.30 621.00	763.30 872.30 621.00		752.20 ± 126.02	752.20 ± 126.02	
½	675.33 627.33 660.83	930.80		654.50 ± 24.62	930.80	
1	535.42 578.33 592.91	644.58		568.89 ± 29.89	644.58	
2	644.16 556.25 636.25	324.58		612.22 ± 48.63	324.58	
4	608.75 632.91 582.50	322.50		608.05 ± 25.21	322.50	
6	568.33 577.50 552.50	840.30		566.11 ± 12.65	840.30	
8	672.08 772.92 637.91	230.83		694.30 ± 70.19	230.83	

TABLE 7

DATA FROM THE THIRD EXPOSURE OF NO_2 GAS TO SOYBEAN PLANTS AT 0.2, 0.5, AND 0.8 ppm

Time (hrs)	CPM Per Gram Wet Tissue			Mean CPM Per Gram Wet Tissue		
	0.2 ppm	0.5 ppm	0.8 ppm	0.2 ppm	0.5 ppm	0.8 ppm
0	786.25 678.75 707.50	786.25 678.75 707.50	786.25 678.75 707.50	724.17 ± 66.65	724.17 ± 55.65	724.17 ± 55.65
½	687.50	372.08 300.83	616.00 649.25	687.50	336.46 ± 50.38	632.62 ± 23.51
1	997.50 617.08 704.16	455.41 416.25 441.83 373.33	626.25 664.25	772.91 ± 199.31	421.71 ± 36.11	645.25 ± 26.87
2	744.58 994.58 666.25	303.75 303.75	425.87 433.62	801.80 ± 171.48	303.75	429.74 ± 5.48
4	581.67	503.33 411.25 529.58	599.50 527.50 563.75	581.67	481.39 ± 62.14	563.58 ± 36.00
6	630.00 597.08 490.83	796.25 696.67 755.83 692.50	591.50 605.50 578.12	572.64 ± 72.74	735.31 ± 49.87	591.71 ± 13.69
8	430.83 327.92	700.41 583.33 655.00 614.07	478.80 470.87 486.80 476.80	379.37 ± 72.77	638.20 ± 50.81	478.32 ± 6.58

TABLE 8

DATA FROM PLANTS RECOVERED FROM NO_2 GAS FUMIGATION AT 0.2, 0.5, AND 0.8 ppm

Time (hrs)	CPM Per Gram Wet Tissue			Mean CPM Per Gram Wet Tissue		
	0.2 ppm	0.5 ppm	0.8 ppm	0.2 ppm	0.5 ppm	0.8 ppm
0	225.00 127.92	225.00 127.92	225.00 127.92	225.00	225.00	225.00
½	1942.08 1840.70	109.17 507.08	1244.60 1317.10	1891.39 ± 71.69	507.08	1280.85 ± 51.26
1	1133.30 1094.58	232.08 543.75	1011.94 898.75	1113.94 ± 27.38	543.75	955.21 ± 79.85
2	927.50 1177.50	567.92 545.42	1369.58 2031.67	1052.50 ± 176.78	556.67 ± 15.00	1369.58
4	944.17 1486.25	123.75 552.08	1662.08 5713.33	1215.21 ± 383.31	552.08	1662.08
6	787.92 1223.75	144.17 339.58	714.17 570.83	1005.84 ± 308.18	339.58	642.50 ± 101.36
8	1030.83 924.17	367.50 281.25	421.25 443.75	977.50 ± 75.42	367.50	432.50 ± 15.91

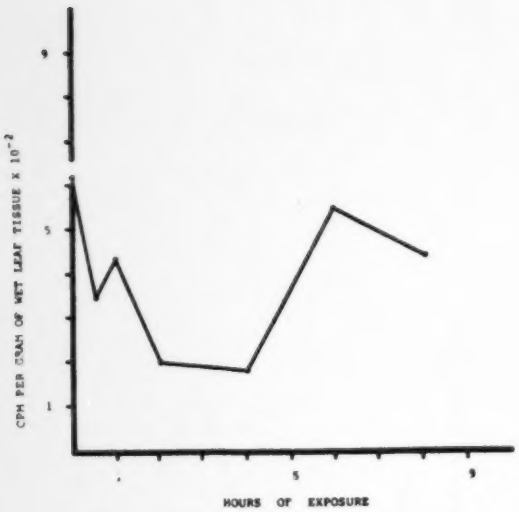

Figure 1a. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.2 ppm, first exposure.

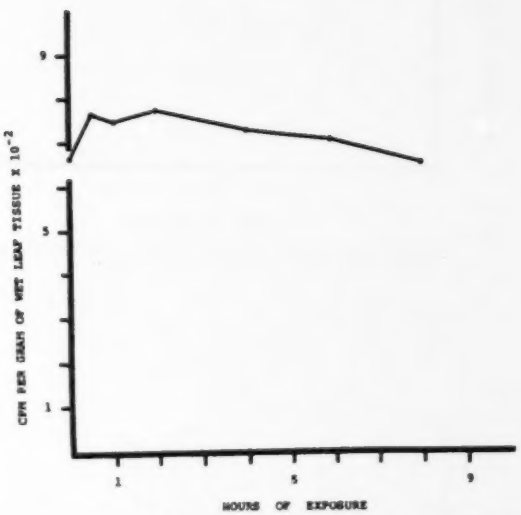

Figure 1b. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.5 ppm, first exposure.

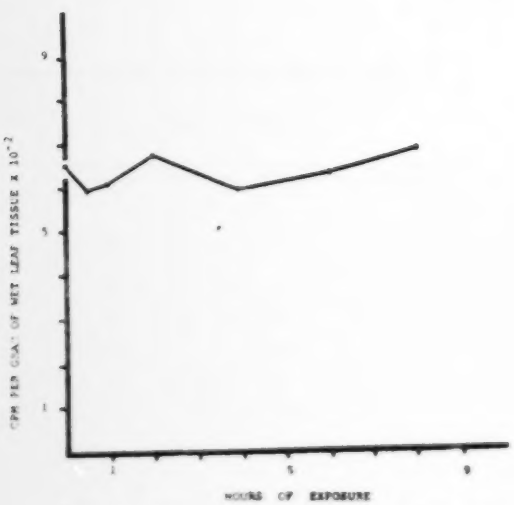

Figure 1c. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.8 ppm, first exposure.

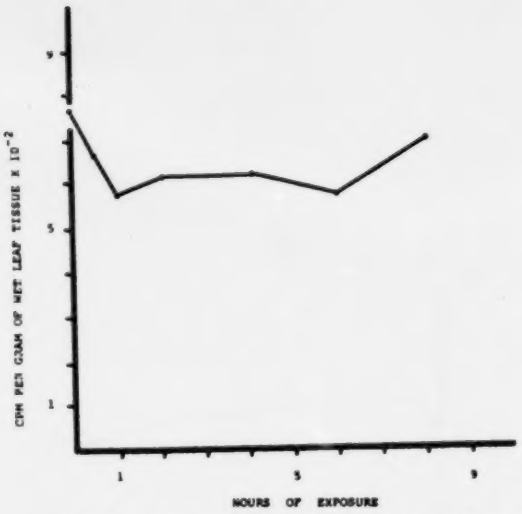

Figure 2a. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.2 ppm, second exposure.

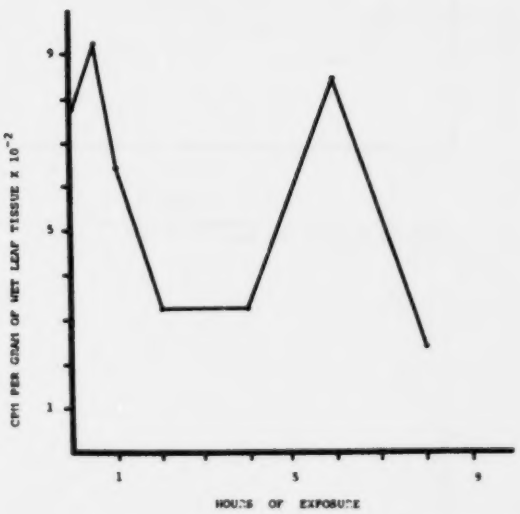

Figure 2b. L-Leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.5 ppm, second exposure.

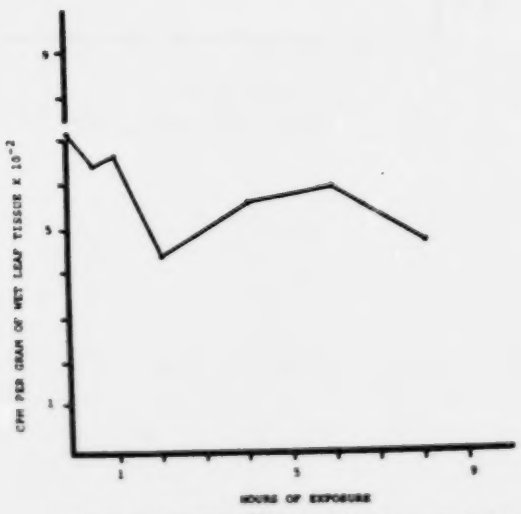

Figure 2c. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.8 ppm, second exposure.

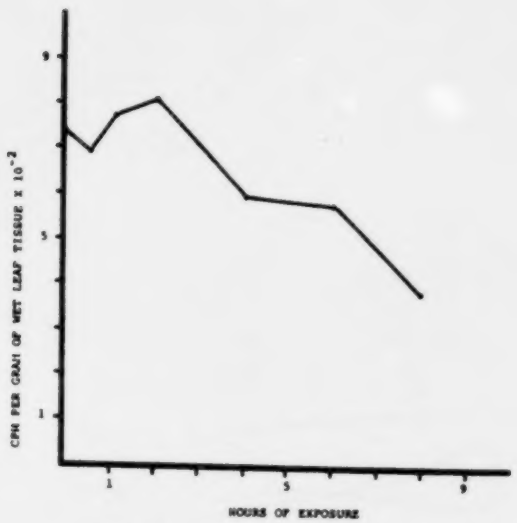

Figure 3a. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.2 ppm, third exposure.

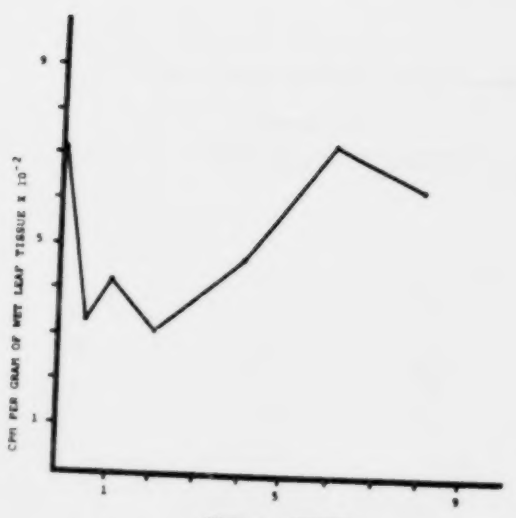

Figure 3b. L-leucine C^{14} incorporation to soybean leaf tissue exposed to NO_2 gas at 0.5 ppm, third exposure.

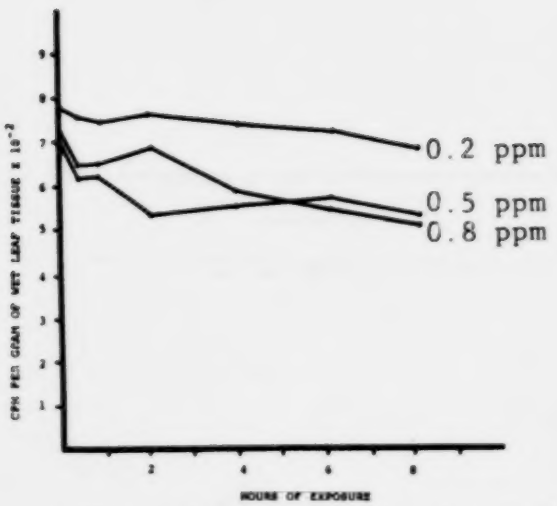

Figure 4. A combined graph of all incorporation of L-leucine C^{14} to soybean leaf tissue at 0.2, 0.5 and 0.8 ppm.

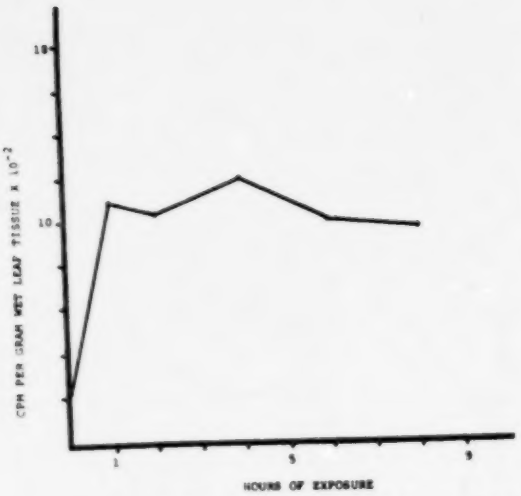

Figure 5a. L-leucine C^{14} incorporation to soybean leaf tissue from plants recovered from 0.2 ppm NO_2 gas.

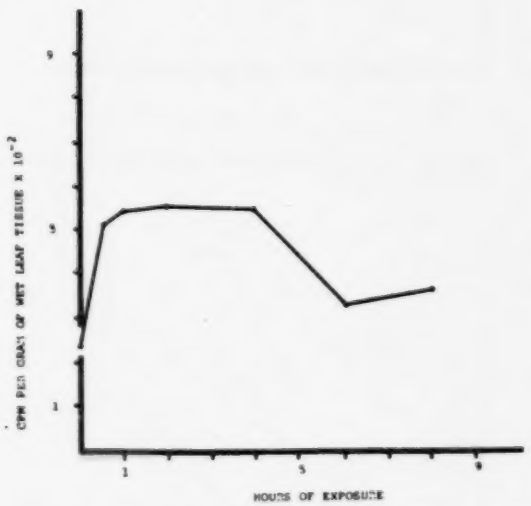

Figure 5b. L-leucine C^{14} incorporation to soybean leaf tissue from plants recovered from 0.5 ppm NO_2 gas.

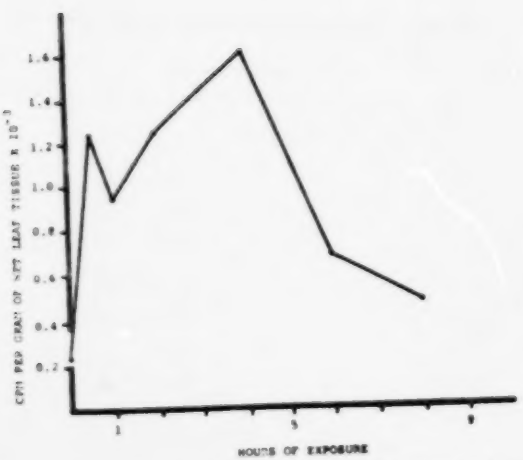

Figure 5c. L-leucine C^{14} incorporation to soybean leaf tissue from plants recovered from 0.8 ppm NO_2 gas.

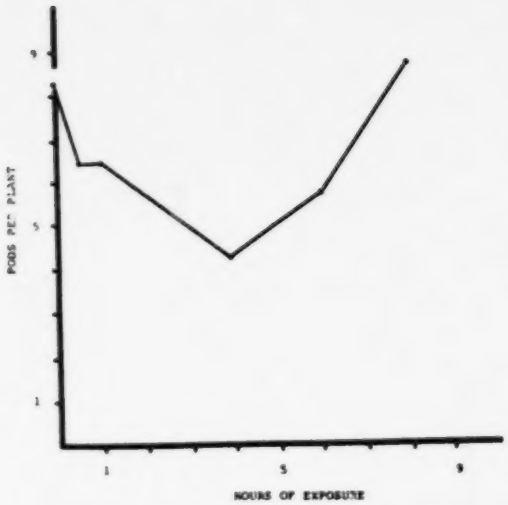

Figure 6a. Yield of plants after 62 days of recovery from 0.2 ppm NO_2 gas.

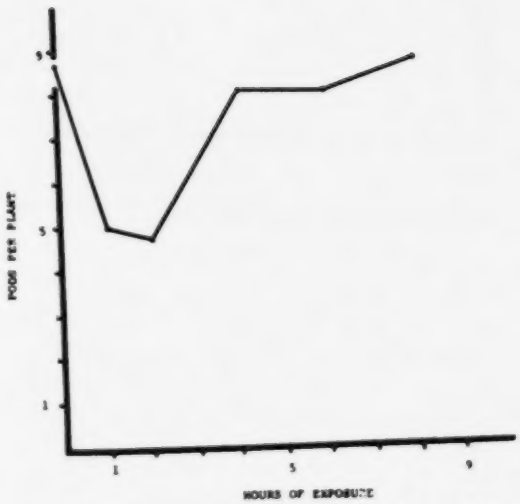

Figure 6b. Yield of plants after 62 days of recovery from 0.5 ppm NO_2 gas.

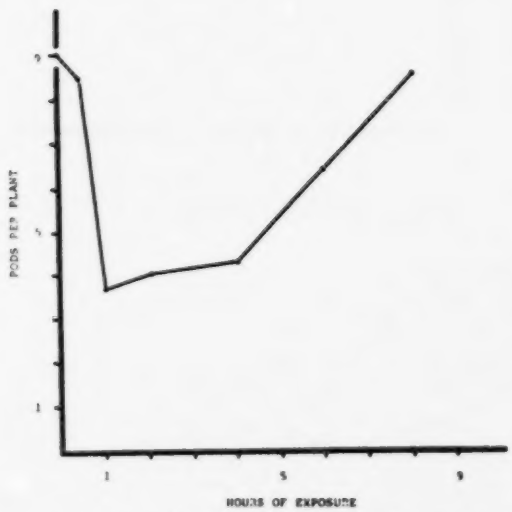

Figure 6c. Yield of plants after 62 days of recovery from 0.8 ppm NO_2 gas.

APPENDIX B

SO_2 EXPOSURE

Tables

1. Leaf plastochron index of soybean plant (age: 30 days), grown under greenhouse conditions (0.2, 195, 0.8, laboratory control and chamber control); exposure 1, 2, and 3 (p. 1-13).

2. a,b,c; Moisture content and dry weight of soybean leaves exposed to SO_2 gas (exp. 1, 2, 3) (p. 14-16).

3. a,b; Dry weights of leaf tissue exposed to SO_2 (statistical analysis) (p. 17).

4. Dry weights of leaf tissue exposed to SO_2, percent moisture content (0.2, 0.5, 0.8 ppm), statistical analysis (p. 18).

5. a,b,c; Total protein content of soybean leaf (mg/gm wet tissue), statistical analysis (p. 19-20).

6. Total protein content of soybean leaf (mg/gm dry tissue), statistical analysis (p. 21).

7. Insoluble protein content of soybean leaf as affected by SO_2 exposure (laboratory and chamber controls, hours exposed, standard, and absorbance) (p.22).

8. Total protein content of soybean leaf at recovery, statistical analysis (SO_2 exposure) (p. 32).

TABLE 1

LEAF PLASTOCHRON INDEX OF SOYBEAN PLANT (AGE: 30 DAYS OLD, GROWN UNDER GREENHOUSE CONDITIONS)

Hours Exposed	Pot No.	LPI VALUES Leaf No. 1	2	3	4
		Exp. 1 - 0.2 ppm			
0	1	0.13	-0.84	-1.88	-2.36
		0.16	-0.88	-1.85	-1.43
	2	0.15	-0.86	-1.82	-2.01
		0.19	-0.85	-1.58	
	3	0.25	-0.82	-1.78	-1.42
		0.10	-0.78	-1.77	
	4	0.21	-0.93	-1.77	-2.46
		0.21	-0.88	-1.91	-2.63
½	1	0.06	-0.96	-1.77	-
		0.23	-0.92	-1.01	
	2	0.21	-0.97	-1.6	-
		0.10	-0.84	-1.51	
	3	0.12	-0.91	-1.73	-
		0.15	-0.80	-1.17	
	4	0.11	-0.90	-1.87	-2.41
		0.10	-0.93	-1.84	-1.87
1	1	0.18	-0.91	-1.57	-
		0.43	-0.75	-1.55	
	2	0.87	-0.83	-1.79	-
		0.18	-0.91	-1.69	
	3	0.18	-0.93	-1.75	
		0.11	-0.92	-1.86	-2.09
	4	0.16	-0.84	-1.54	
		0.09	-0.96	-1.93	-2.01
2	1	0.04	-0.86	-1.89	-2.26
		0.04	-0.89	-1.88	-2.16
	2	0.15	-0.91	-2.01	-2.34
		0.15	-0.98	-1.81	-1.6
	3	0.06	-0.97	-1.95	2.65
		0.05	-0.91	-1.96	-2.6
	4	0.11	-0.81	-1.29	-
		0.11	-0.95	-1.61	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 1 - 0.2 ppm (Continued)			
4	1	0.15	-0.95	-1.69	-0.98
		0.09	-0.98	-1.71	
	2	0.19	-0.84	-1.92	-1.35
		0.14	-0.77	-1.76	
	3	0.07	-0.79	-1.76	-
		0.19	-0.91	-1.76	
	4	0.16	-0.80	-1.76	-
		0.18	-0.80	-1.41	
6	1	0.13	-0.92	-1.78	-1.91
		0.16	-0.87	-1.60	
	2	0.09	-0.93	-1.62	-
		0.16	-0.81	-1.56	
	3	0.17	-0.76	-1.78	-
		0.17	-0.76	-1.55	
	4	0.12	-0.98	-1.94	-2.6
		0.16	-0.76	-1.60	
8	1	0.15	-0.87	-1.60	-
		0.19	-0.77	-1.30	
	2	0.19	-0.73	-1.51	
		0.16	-0.87	-1.81	-1.19
	3	0.13	-0.90	-1.56	
		0.14	-0.94	-1.84	-2.20
	4	0.15	-0.89	-1.65	-
		0.15	-0.93	-1.48	
		Exp. 1 - 0.5 ppm			
0	1	0.18	-0.94	-1.94	-2.67
		0.15	-0.92	-1.95	-2.47
	2	0.27	-0.85	-1.76	-1.35
		0.22	-0.87	-1.85	
	3	0.21	-0.89	-1.67	
		0.14	-0.94	-1.91	-2.55
	4	0.18	-0.89	-1.92	-2.49
		0.18	-0.91	-1.86	-2.47

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 1 - 0.5 ppm (continued)			
½	1	0.22	-0.87	-1.74	
		0.27	-0.81	-1.76	-1.87
	2	0.25	-0.89	-1.84	-2.06
		0.17	-0.74	-1.82	
	3	0.21	-0.85	-1.80	
		0.18	-0.86	-1.77	-0.98
	4	0.18	-0.87	-1.82	-2.11
		0.22	-0.89	-1.87	-2.00
1	1	0.16	-0.88	-1.91	-2.6
		0.27	-0.87	-1.87	-2.26
	2	0.20	-0.90	-1.99	-2.37
		0.19	-0.90	-1.87	-2.36
	3	0.18	-0.86	-1.67	-0.40
		0.22	-0.93	-1.93	-1.95
	4	0.20	-0.85	-1.64	
		0.22	-0.88	-1.83	-2.04
2	1	0.12	-0.85	-1.85	-2.18
		0.15	-0.93	-1.95	-2.26
	2	0.21	-0.92	-1.89	-1.35
		0.16	-0.94	-1.92	-1.91
	3	0.18	-0.92	-1.83	
		0.16	-0.91	-1.92	-2.29
	4	0.16	-0.89	-1.91	-2.37
		0.24	-0.87	-1.91	-0.86
4	1	0.19	-0.84	-1.86	-2.36
		0.19	-0.87	-1.89	
	2	0.20	-0.93	-1.89	
		0.13	-0.91	-1.92	-2.29
	3	0.20	-0.85	-1.90	-2.68
		0.43	-0.71	-1.11	
	4	0.27	-0.85	-1.80	-1.61
		0.24	-0.83	-1.85	-2.36

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 1 - 0.5 ppm (Continued)			
6	1	0.16	-0.77	-1.88	-2.34
		0.15	-0.76	-1.84	-2.24
	2	0.26	-0.85	-1.85	
		0.33	-0.83	-1.83	-2.38
	3	0.29	-0.87	-1.88	
		0.24	-0.85	-1.81	-1.71
	4	0.24	-0.85	-1.69	-1.42
		0.19	-0.84	-1.89	
8	1	0.24	-0.90	-1.69	-0.86
		0.18	-0.89	-1.77	
	2	0.23	-0.90	-1.96	-2.6
		0.17	-0.94	-1.94	
	3	0.18	-0.91	-1.93	-2.55
		0.25	-0.86	-1.87	-2.36
	4	0.20	-0.91	-1.87	
		0.23	-0.88	-1.89	-2.5
		Exp. 1 - 0.8 ppm			
0	1	0.19	-0.80	-1.53	-0.40
		0.21	-0.80	-1.09	
	2	0.12	-0.89	-1.73	-1.35
		0.24	-0.58	-0.02	
	3	0.24	-0.87	-1.87	-2.20
		0.13	-0.87	-1.18	
	4	0.26	-0.80	-1.55	
		0.19	-0.85	-1.72	-1.35
½	1	0.11	-0.90	-1.70	-0.19
		0.18	-0.81	-1.76	-1.09
	2	0.12	-0.96	-1.92	
		0.15	-0.88	1.74	-1.61
	3	0.17	-0.81	-1.37	-
		0.19	-0.72	-1.14	
	4	0.13	-0.87	-1.54	-2.29
		0.18	-0.87	-1.93	-2.16

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 1 - 0.8 ppm (Continued)			
1	1	0.15	-0.88	-1.91	-2.50
		0.27	-0.87	-1.89	
	2	0.19	-0.87	-1.85	-1.94
		0.19	-0.87	-1.73	
	3	0.21	-0.90	-1.92	-2.64
		0.24	-0.90	-1.89	-1.19
	4	0.28	-0.81	-1.56	-1.19
		0.28	-0.81	-1.77	
2	1	0.30	-0.82	-1.79	-0.98
		0.19	-0.87	-1.91	-2.37
	2	0.27	-0.83	-1.80	
		0.32	-0.87	-1.81	-2.01
	3	0.22	-0.82	-1.16	-1.82
		0.15	-0.87	-1.75	
	4	0.23	-0.87	-1.88	-1.71
		0.20	-0.87	-1.84	-1.71
4	1	0.26	-0.75	-1.87	-2.06
		0.11	-0.80	-1.94	-1.35
	2	0.19	-0.67	-1.87	-1.35
		0.35	-0.82	-1.77	-1.88
	3	0.24	-0.87	-1.66	
		0.20	-0.84	-1.84	-2.36
	4	0.31	-0.82	-1.80	-1.18
		0.29	-0.84	-1.89	
6	1	0.33	-0.76	-1.77	-0.73
		0.24	-0.85	-1.84	
	2	0.16	-0.90	-1.80	-1.19
		0.24	-0.81	-1.24	
	3	0.24	-0.89	-1.86	-2.41
		0.26	-0.76	-1.72	
	4	0.20	-0.86	-1.91	-2.04
		0.24	-0.88	-1.90	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 1 - 0.8 ppm (Continued)			
8	1	0.18	-0.82	-1.81	-2.00
		0.24	-0.80	-1.74	
	2	0.20	-0.97	-1.88	-2.33
		0.24	-0.70	-1.92	-2.18
	3	0.16	-0.87	-1.90	-2.50
		0.17	-0.87	-1.92	
	4	0.26	-0.86	-1.82	-
		0.38	-0.76	-1.73	
		Chamber Control			
4	1	0.19	-0.91	-1.52	-1.42
		0.16	-0.85	-1.14	
	2	0.16	-0.82	-1.28	-1.19
		0.13	-0.81	-0.76	
	3	0.17	-0.85	-1.10	-1.42
		0.15	-0.84	-0.95	-1.19
	4	0.16	-0.87	-1.34	-
		0.19	-0.83	-1.45	
6	1	0.18	-0.85	-1.04	-0.03
		0.15	-0.75	-1.40	
	2	0.19	-0.81	-1.39	-1.88
		0.20	-0.88	-1.33	-1.71
	3	0.20	-0.86	-1.67	
		0.19	-0.84	-1.58	-0.86
	4	0.20	-0.85	-1.46	-0.73
		0.18	-0.83	-1.72	-0.86
8	1	0.16	-0.90	-1.56	-
		0.15	-0.86	-1.01	
	2	0.16	-0.86	-1.63	-1.66
		0.20	-0.89	-1.64	-2.04
	3	0.18	-0.82	-1.18	
		0.15	-0.85	-1.01	-1.09
	4	0.24	-0.81	-1.68	
		0.16	-0.84	-1.50	-1.09

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.2 ppm			
0	1	0.13	-0.80	-1.87	-1.91
		0.17	-0.87	-1.83	-1.35
	2	0.17	-0.85	-1.81	-1.88
		0.20	-0.84	-1.58	-0.86
	3	0.25	-0.79	-1.77	-0.86
		0.10	-0.78	-1.77	-0.86
	4	0.19	-0.91	-1.86	-2.35
		0.20	-0.88	-1.90	-2.62
½	1	0.07	-0.94	-1.80	-0.86
		0.13	-0.91	-1.36	
	2	0.21	-0.87	-1.55	-
		0.10	-0.84	-1.48	
	3	0.12	-0.91	-1.72	-0.86
		0.15	-0.80	-1.26	
	4	0.12	-0.90	-1.87	-2.26
		0.10	-0.91	-1.84	-1.79
1	1	0.17	-0.91	-1.59	
		0.17	-0.76	-1.57	
	2	0.09	-0.83	-1.79	-
		0.18	-0.89	-1.62	
	3	0.17	-0.93	-1.77	
		0.11	-0.91	-1.86	-2.06
	4	0.16	-0.85	-1.55	
		0.08	-0.96	-1.94	-1.95
2	1	0.05	-0.87	-1.88	-2.04
		0.05	-0.84	-1.85	-2.16
	2	0.16	-0.91	-2.00	-2.26
		0.15	-0.94	-1.79	-1.55
	3	0.08	-0.95	-1.94	-1.18
		0.07	-0.89	-1.95	-2.44
	4	0.13	-0.82	-1.39	-
		0.13	-0.94	-1.55	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.2 ppm (Continued)			
4	1	0.15	-0.94	-1.70	-0.86
		0.10	-0.95	-1.72	
	2	0.20	-0.85	-1.89	-1.35
		0.14	-0.77	-1.78	
	3	0.08	-0.81	-1.77	
		0.19	-0.91	-1.76	-1.66
	4	0.17	-0.79	-1.78	-
		0.17	-0.82	-1.31	
6	1	0.12	-0.91	-1.77	-1.88
		0.16	-0.88	-1.62	
	2	0.09	-0.94	-1.63	-
		0.16	-0.82	-1.60	
	3	0.17	-0.77	-1.77	-
		0.17	-0.77	-1.54	
	4	0.14	-0.95	-1.91	-2.03
		0.16	-0.80	-1.58	
8	1	0.14	-0.87	-1.63	-
		0.17	-0.79	-1.36	
	2	0.19	-0.70	-1.58	-1.09
		0.16	-0.87	-1.79	
	3	0.12	-0.91	-1.60	
		0.13	-0.93	-1.83	-1.76
	4	0.14	-0.90	-1.63	-
		0.14	-0.92	-1.45	
		Exp. 2 - 0.5 ppm			
0	1	0.20	-0.93	-1.92	-2.26
		0.19	-0.91	-1.97	
	2	0.27	-0.87	-1.79	
		0.20	-0.89	-1.85	-1.19
	3	0.21	-0.91	-1.73	
		0.16	-0.93	-1.91	-2.40
	4	0.18	-0.89	-1.89	-1.94
		0.19	-0.90	-1.85	-2.34

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.5 ppm (Continued)			
½	1	0.25	-0.86	-1.68	-
		0.28	-0.77	-1.73	
	2	0.25	-0.89	-1.85	-1.85
		0.16	-0.75	-1.83	
	3	0.20	-0.87	-1.84	
		0.18	-0.89	-1.78	-1.35
	4	0.18	-0.87	-1.82	-1.91
		0.21	-0.89	-1.86	
1	1	0.13	-0.89	-1.92	-2.30
		0.25	-0.86	-1.87	-1.76
	2	0.18	-0.87	-1.69	-0.98
		0.21	-0.92	-1.89	-1.91
	3	0.16	-0.85	-1.63	
		0.21	-0.87	-1.82	-1.19
	4	0.20	-0.85	-1.63	
		0.20	-0.88	-1.79	-1.91
2	1	0.13	-0.86	-1.85	-2.16
		0.15	-0.91	-1.93	
	2	0.21	-0.88	-1.83	-1.19
		0.15	-0.93	-1.89	
	3	0.18	-0.91	-1.82	
		0.15	-0.88	-1.92	-2.03
	4	0.16	-0.88	-1.90	-0.86
		0.24	-0.85	-1.89	-0.86
4	1	0.20	-0.79	-1.84	-2.18
		0.18	-0.88	-1.88	
	2	0.16	-0.93	-1.89	
		0.13	-0.89	-1.92	-1.09
	3	0.20	-0.84	-1.89	-2.43
		0.35	-0.74	-1.69	
	4	0.25	-0.82	-1.76	-1.27
		0.20	-0.84	-1.85	-1.19

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.5 ppm (Continued)			
6	1	0.12	-0.81	-1.90	-2.36
		0.18	-0.79	-1.87	-2.09
	2	0.25	-0.85	-1.83	
		0.33	-0.85	-1.84	-2.40
	3	0.24	-0.87	-1.88	-
		0.21	-0.87	-1.84	
	4	0.20	-0.87	-1.69	-1.42
		0.16	-0.85	-1.90	
8	1	0.19	-0.91	-1.71	
		0.19	-0.90	-1.77	-1.09
	2	0.20	-0.92	-1.94	-2.36
		0.18	-0.92	-1.92	
	3	0.18	-0.92	-1.94	-2.26
		0.25	-0.86	-1.87	-1.09
	4	0.20	-0.91	-1.87	
		0.22	-0.87	-1.88	-2.48
		Exp. 2 - 0.8 ppm			
0	1	0.18	-0.81	-1.58	-
		0.19	-0.82	-1.30	
	2	0.10	-0.89	-1.76	-1.42
		0.20	-0.71	-1.74	
	3	0.21	-0.87	-1.86	-
		0.13	-0.89	-1.45	
	4	0.25	-0.84	-1.55	-
		0.19	-0.86	-1.70	
½	1	0.13	-0.89	-1.76	-0.86
		0.17	-0.82	-1.76	-0.98
	2	0.11	-0.95	-1.92	-
		0.17	-0.87	-1.76	
	3	0.18	-0.80	-1.61	-
		0.20	-0.76	-1.45	
	4	0.13	-0.88	-1.58	-
		0.16	-0.88	-1.92	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.8 ppm (Continued)			
1	1	0.21	-0.87	-1.91	-
		0.23	-0.87	-1.90	
	2	0.19	-0.87	-1.86	-1.91
		0.19	-0.87	-1.76	
	3	0.20	-0.91	-1.92	-2.34
		0.22	-0.90	-1.90	-0.86
	4	0.25	-0.82	-1.45	-
		0.27	-0.80	-1.79	
2	1	0.26	-0.82	-1.79	
		0.16	-0.89	-1.90	-1.91
	2	0.24	-0.83	-1.79	
		0.27	-0.87	-1.84	-2.16
	3	0.22	-0.84	-1.58	-
		0.13	-0.88	-1.80	
	4	0.22	-0.86	-1.87	
		0.16	-0.89	-1.85	-2.04
4	1	0.25	-0.76	-1.88	-1.91
		0.12	-0.84	-1.94	
	2	0.23	-0.90	-1.87	
		0.18	-0.84	-1.87	-2.26
	3	0.29	-0.80	-1.84	-
		0.29	-0.84	-1.89	
	4	0.26	-0.80	-1.81	-
		0.24	-0.84	-1.90	
6	1	0.29	-0.76	-1.70	-
		0.20	-0.87	-1.86	
	2	0.14	-0.92	-1.84	-2.34
		0.20	-0.83	-1.45	
	3	0.19	-0.90	-1.83	
		0.24	-0.77	-1.74	-2.26
	4	0.18	-0.88	-1.89	-2.16
		0.21	-0.90	-1.73	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 2 - 0.8 ppm (Continued)			
8	1	0.16	-0.82	-1.79	-1.95
		0.20	-0.80	-1.74	
	2	0.20	-0.95	-1.88	-2.26
		0.20	-0.75	-1.87	
	3	0.17	-0.88	-1.90	-1.09
		0.21	-0.87	-1.92	
	4	0.24	-0.87	-1.82	-
		0.28	-0.79	-1.75	
		Chamber Control			
4	1	0.18	-0.90	-1.58	-1.27
		0.14	-0.85	-1.31	
	2	0.16	-0.82	-1.57	-1.19
		0.13	-0.81	-0.80	
	3	0.18	-0.87	-1.64	-1.27
		0.13	-0.85	-1.57	-0.86
	4	0.14	-0.88	-1.57	-
		0.18	-0.84	-1.31	
6	1	0.19	-0.84	-1.16	-0.86
		0.15	-0.76	-1.43	
	2	0.19	-0.82	-1.58	-1.55
		0.20	-0.87	-1.45	-1.55
	3	0.19	-0.87	-1.71	
		0.19	-0.83	-1.64	-0.86
	4	0.18	-0.84	-1.49	-0.86
		0.18	-0.84	-1.76	
8	1	0.18	-0.84	-1.76	-1.09
		0.16	-0.91	-1.64	
	2	0.14	-0.87	-1.61	-
		0.16	-0.86	-1.65	
	3	0.20	-0.85	-1.65	-
		0.18	-0.81	-1.52	
	4	0.13	-0.84	-1.34	-2.29
		0.16	-0.83	-1.69	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.2 ppm			
0	1	0.13	-0.83	-1.86	-2.26
		0.17	-0.87	-1.83	-1.19
	2	0.16	-0.85	-1.81	-1.49
		0.18	-0.84	-1.66	
	3	0.24	-0.84	-1.76	
		0.12	-0.80	-1.75	-1.19
	4	0.20	-0.91	-1.87	-2.34
		0.20	-0.85	-1.89	-1.19
½	1	0.12	-0.92	-1.76	-
		0.11	-0.91	-1.66	
	2	0.20	-0.86	-1.65	
		0.10	-0.87	-1.64	-1.19
	3	0.11	-0.90	-1.76	-
		0.14	-0.82	-1.61	
	4	0.13	-0.90	-1.88	-2.37
		0.11	-0.92	-1.86	-1.80
1	1	0.18	-0.89	-1.66	-
		0.16	-0.78	-1.61	
	2	0.09	-0.84	-1.82	-
		0.18	-0.89	-1.63	
	3	0.16	-0.91	-1.80	-1.71
		0.11	-0.86	-1.86	
	4	0.14	-0.85	-1.66	
		0.10	-0.94	-1.91	-2.04
2	1	0.07	-0.86	-1.87	-2.01
		0.06	-0.90	-1.87	-2.18
	2	0.17	-0.88	-1.95	
		0.13	-0.96	-1.84	-2.01
	3	0.08	-0.95	-1.93	-2.41
		0.05	-0.91	-1.92	
	4	0.10	-0.84	-1.52	-
		0.10	-0.92	-1.64	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.2 ppm (Continued)			
4	1	0.13	-0.92	-1.76	
		0.10	-0.94	-1.76	-2.26
	2	0.16	-0.82	-1.87	-1.42
		0.14	-0.80	-1.82	
	3	0.08	-0.84	-1.81	-
		0.16	-0.89	-1.78	
	4	0.13	-0.82	-1.75	-
		0.19	-0.82	-1.65	
6	1	0.10	-0.88	-1.80	-2.04
		0.16	-0.87	-1.68	
	2	0.07	-0.91	-1.65	
		0.16	-0.84	-1.69	-2.37
	3	0.18	-0.80	-1.82	-
		0.16	-0.80	-1.62	
	4	0.10	-0.93	-1.93	-2.37
		0.13	-0.83	-1.66	
8	1	0.13	-0.86	-1.66	-
		0.21	-0.78	-1.68	
	2	0.18	-0.76	-1.59	
		0.15	-0.87	-1.84	-1.55
	3	0.10	-0.90	-1.69	-
		0.13	-0.90	-1.85	
	4	0.13	-0.89	-1.71	
		0.15	-0.91	-1.68	-2.20
		Exp. 3 - 0.5 ppm			
0	1	0.16	-0.91	-1.91	-2.26
		0.16	-0.92	-1.92	-2.16
	2	0.18	-0.86	-1.78	-0.98
		0.20	-0.88	-1.85	
	3	0.21	-0.89	-1.73	
		0.16	-0.91	-1.92	-2.37
	4	0.18	-0.90	-1.93	-2.47
		0.18	-0.91	-1.85	-2.36

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.5 ppm (Continued)			
½	1	0.22	-0.87	-1.76	
		0.24	-0.84	-1.84	-2.34
	2	0.24	-0.89	-1.85	-1.76
		0.16	-0.75	-1.82	
	3	0.20	-0.84	-1.81	
		0.18	-0.84	-1.78	-1.19
	4	0.16	-0.88	-1.81	-1.91
		0.22	-0.90	-1.88	-2.34
1	1	0.15	-0.87	-1.90	-2.56
		0.24	-0.87	-1.88	-2.34
	2	0.20	-0.89	-1.93	
		0.21	-0.89	-1.87	-2.47
	3	0.18	-0.87	-1.71	-1.19
		0.20	-0.91	-1.92	-1.66
	4	0.16	-0.83	-1.76	
		0.20	-0.88	-1.84	-1.91
2	1	0.13	-0.84	-1.86	-2.16
		0.16	-0.90	-1.93	-2.18
	2	0.21	-0.89	-1.80	-1.42
		0.16	-0.92	-1.93	-2.16
	3	0.16	-0.90	-1.83	
		0.16	-0.90	-1.92	-2.18
	4	0.16	-0.88	-1.90	-2.11
		0.20	-0.87	-1.90	
4	1	0.19	-0.79	-1.81	-2.34
		0.19	-0.86	-1.84	
	2	0.18	-0.92	-1.90	
		0.12	-0.90	-1.92	-2.26
	3	0.16	-0.87	-1.90	-2.34
		0.22	-0.86	-1.72	
	4	0.23	-0.84	-1.82	-1.55
		0.19	-0.83	-1.84	-2.20

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.5 ppm (Continued)			
½	1	0.22	-0.87	-1.76	
		0.24	-0.84	-1.84	-2.34
	2	0.24	-0.89	-1.85	-1.76
		0.16	-0.75	-1.82	
	3	0.20	-0.84	-1.81	
		0.18	-0.84	-1.78	-1.19
	4	0.16	-0.88	-1.81	-1.91
		0.22	-0.90	-1.88	-2.34
1	1	0.15	-0.87	-1.90	-2.56
		0.24	-0.87	-1.88	-2.34
	2	0.20	-0.89	-1.93	
		0.21	-0.89	-1.87	-2.47
	3	0.18	-0.87	-1.71	-1.19
		0.20	-0.91	-1.92	-1.66
	4	0.16	-0.83	-1.76	
		0.20	-0.88	-1.84	-1.91
2	1	0.13	-0.84	-1.86	-2.16
		0.16	-0.90	-1.93	-2.18
	2	0.21	-0.89	-1.80	-1.42
		0.16	-0.92	-1.93	-2.16
	3	0.16	-0.90	-1.83	
		0.16	-0.90	-1.92	-2.18
	4	0.16	-0.88	-1.90	-2.11
		0.20	-0.87	-1.90	
4	1	0.19	-0.79	-1.81	-2.34
		0.19	-0.86	-1.84	
	2	0.18	-0.92	-1.90	
		0.12	-0.90	-1.92	-2.26
	3	0.16	-0.87	-1.90	-2.34
		0.22	-0.86	-1.72	
	4	0.23	-0.84	-1.82	-1.55
		0.19	-0.83	-1.84	-2.20

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.5 ppm (Continued)			
6	1	0.16	-0.80	-1.87	-2.26
		0.17	-0.76	-1.84	-2.31
	2	0.16	-0.85	-1.84	
		0.24	-0.82	-1.84	-2.28
	3	0.22	-0.87	-1.89	
		0.19	-0.85	-1.82	-1.80
	4	0.19	-0.85	-1.72	-1.66
		0.16	-0.87	-1.87	
8	1	0.19	-0.89	-1.70	-1.19
		0.20	-0.84	-1.80	
	2	0.19	-0.89	-1.92	-2.44
		0.18	-0.91	-1.92	
	3	0.16	-0.90	-1.92	-2.43
		0.24	-0.84	-1.88	-2.34
	4	0.16	-0.90	-1.88	
		0.18	-0.87	-1.88	-2.37
		Exp. 3 - 0.8 ppm			
0	1	0.16	-0.82	-1.63	-0.86
		0.18	-0.84	-1.65	
	2	0.13	-0.88	-1.76	-1.49
		0.19	-0.73	-1.63	
	3	0.19	-0.90	-1.87	-2.34
		0.13	-0.86	-1.70	
	4	0.24	-0.84	-1.73	
½	1	0.11	-0.90	-1.76	-1.19
		0.16	-0.82	-1.77	-0.86
	2	0.11	-0.95	-1.91	
		0.16	-0.87	-1.76	-1.66
	3	0.16	-0.82	-1.76	-
		0.16	-0.76	-1.65	
	4	0.13	-0.88	-1.72	-2.16
		0.16	-0.87	-1.91	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.8 ppm (Continued)			
1	1	0.15	-0.87	-1.90	-2.43
		0.24	-0.88	-1.90	
	2	0.16	-0.87	-1.88	-
		0.16	-0.88	-1.76	
	3	0.20	-0.90	-1.91	-2.66
		0.20	-0.90	-1.91	
	4	0.24	-0.82	-1.72	-1.09
		0.24	-0.81	-1.81	
2	1	0.24	-0.82	-1.80	-1.19
		0.18	-0.87	-1.90	-2.39
	2	0.22	-0.84	-1.83	-1.80
		0.21	-0.87	-1.80	-1.66
	3	0.19	-0.85	-1.72	-1.60
		0.21	-0.84	-1.74	-1.66
	4	0.19	-0.90	-1.92	-1.19
		0.19	-0.87	-1.84	
4	1	0.24	-0.80	-1.87	-
		0.13	-0.84	-1.93	
	2	0.18	-0.76	-1.86	-
		0.27	-0.82	-1.79	
	3	0.20	-0.88	-1.85	-
		0.19	-0.84	-1.87	-2.26
	4	0.24	-0.81	-1.81	-0.98
		0.24	-0.85	-1.86	
6	1	0.27	-0.80	-1.79	
		0.20	-0.84	-1.85	-1.35
	2	0.15	-0.91	-1.83	-1.19
		0.24	-0.83	-1.63	
	3	0.18	-0.89	-1.86	-2.26
		0.24	-0.74	-1.76	
	4	0.19	-0.87	-1.89	-1.76
		0.21	-0.85	-1.77	

TABLE 1 (Continued)

Hours Exposed	Pot No.	Leaf No. 1	2	3	4
		Exp. 3 - 0.8 ppm (Continued)			
8	1	0.19	-0.84	-1.79	-1.80
		0.21	-0.82	-1.66	
	2	0.19	-0.91	-1.84	-
		0.18	-0.76	-1.70	
	3	0.13	-0.88	-1.84	-2.37
		0.19	-0.86	-1.90	
	4	0.16	-0.87	-1.81	-
		0.22	-0.80	-1.69	
4	1	0.16	-0.90	-1.65	-1.66
		0.17	-0.84	-1.68	
	2	0.16	-0.82	-1.65	-0.86
		0.16	-0.82	-1.58	
	3	0.17	-0.82	-1.68	
		0.16	-0.84	-1.61	-0.98
	4	0.16	-0.87	-1.69	
		0.18	-0.84	-1.44	-1.42
6	1	0.18	-0.83	-1.57	-
		0.16	-0.76	-1.49	
	2	0.20	-0.82	-1.49	-1.91
		0.21	-0.87	-1.60	-1.35
	3	0.20	-0.87	-1.68	
		0.16	-0.84	-1.62	-0.86
	4	0.18	-0.84	-1.61	-0.73
		0.19	-0.83	-1.75	
8	1	0.18	-0.88	-1.57	
		0.16	-0.83	-1.44	
	2	0.16	-0.86	-1.64	-0.86
		0.19	-0.86	-1.69	-1.66
	3	0.21	-0.83	-1.49	-0.98
		0.16	-0.86	-1.61	
	4	0.19	-0.86	-1.72	-
		0.21	-0.82	-1.69	

TABLE 2a

MOISTURE CONTENT AND DRY WEIGHT OF SOYBEAN LEAVES EXPOSED TO SO_2 GAS

Experiment 1

ppm	Time (hrs)	1 gm Leaf w/dish Wt	Dry Leaf w/dish Wt	Moisture Wt	Dry Leaf Wt
0.2	Control(Lab)	2.190	1.438	0.752	0.248
	½	2.193	1.438	0.755	0.245
	1	2.117	1.347	0.770	0.230
	2	2.111	1.336	0.775	0.225
	4	2.211	1.431	0.780	0.220
	6	2.182	1.437	0.745	0.255
	8	2.167	1.427	0.740	0.260
0.5	Control(Lab)	2.406	1.657	0.749	0.251
	½	2.407	1.628	0.749	0.251
	1	2.407	1.648	0.781	0.219
	2	2.440	1.665	0.775	0.225
	4	2.440	1.671	0.769	0.231
	6	2.413	1.673	0.740	0.260
	8	2.404	1.669	0.735	0.265
0.8	Control(Lab)	2.412	1.660	0.752	0.248
	½	2.323	1.622	0.701	0.299
	1	2.315	1.655	0.660	0.340
	2	2.302	1.662	0.640	0.360
	4	2.312	1.692	0.620	0.380
	6	2.328	1.832	0.496	0.504
	8	2.308	1.818	0.490	0.510
---	Control* (Chamber)	2.095	1.348	0.747	0.253
		2.237	1.476	0.751	0.249
---		2.130	1.380	0.750	0.250

*The plants were exposed for 4, 6, and 8 hrs to air in the chamber and tabulated serially in this column.

TABLE 2b

MOISTURE CONTENT AND DRY WEIGHT OF SOYBEAN LEAVES EXPOSED TO SO_2 GAS

Experiment 2

ppm	Time (hrs)	1 gm Leaf w/dish Wt	Dry Leaf w/dish Wt	Moisture Wt	Dry Leaf Wt
0.2	Control(Lab)	2.16	1.42	0.74	0.26
	½	2.192	1.442	0.75	0.25
	1	2.116	1.366	0.75	0.25
	2	2.413	1.663	0.75	0.25
	4	2.16	1.388	0.772	0.228
	6	2.33	1.59	0.74	0.26
	8	2.314	1.58	0.734	0.266
0.5	Control(Lab)	2.312	1.562	0.75	0.25
	½	2.420	1.644	0.776	0.224
	1	2.432	1.662	0.77	0.23
	2	2.150	1.378	0.772	0.228
	4	2.162	1.402	0.76	0.24
	6	2.412	1.667	0.745	0.255
	8	2.36	1.628	0.732	0.268
0.8	Control(Lab)	2.326	1.581	0.745	0.255
	½	2.421	1.771	0.650	0.255
	1	2.171	1.541	0.630	0.37
	2	2.23	1.62	0.61	0.39
	4	2.18	1.58	0.60	0.40
	6	2.182	1.702	0.48	0.52
	8	2.22	1.767	0.45	0.547
---	Control* (Chamber)	2.419	1.679	0.74	0.26
		2.163	1.413	0.75	0.25
		2.222	1.47	0.752	0.248

*The plants were exposed for 4, 6, and 8 hrs to air in the chamber and tabulated serially in this column.

TABLE 2c

MOISTURE CONTENT AND DRY WEIGHT OF SOYBEAN LEAVES EXPOSED TO SO_2 GAS

Experiment 3

ppm	Time (hrs)	1 gm Leaf w/dish Wt	Dry Leaf w/dish Wt	Moisture Wt	Dry Leaf Wt
0.2	Control(Lab)	2.180	1.430	0.750	0.250
	½	2.320	1.560	0.760	0.240
	1	2.410	1.645	0.765	0.235
	2	2.401	1.621	0.780	0.220
	4	2.190	1.405	0.785	0.215
	6	2.311	1.561	0.750	0.250
	8	2.406	1.658	0.748	0.252
0.5	Control(Lab)	2.379	1.639	0.740	0.260
	½	2.192	1.432	0.76	0.24
	1	2.288	1.526	0.762	0.238
	2	2.259	1.509	0.750	0.25
	4	2.319	1.569	0.75	0.25
	6	2.194	1.464	0.730	0.27
	8	2.169	1.429	0.74	0.26
0.8	Control(Lab)	2.259	1.517	0.742	0.258
	½	2.221	1.511	0.71	0.29
	1	2.23	1.558	0.672	0.328
	2	2.260	1.630	0.630	0.370
	4	2.271	1.650	0.625	0.375
	6	2.180	1.69	0.49	0.51
	8	2.339	1.854	0.485	0.515
---	Control* (Chamber)	2.401	1.651	0.75	0.25
		2.394	1.654	0.74	0.26
		2.278	1.537	0.741	0.259

*The plants were exposed for 4, 6, and 8 hrs to air in the chamber and tabulated serially in this column.

TABLE 3a

DRY WEIGHTS OF ONE GRAM EACH LEAF TISSUE AFTER SO_2 GAS FUMIGATION

A. O. V

Source	d.f.	SS	MS	f	P
Total	72	582.299			
Mean-1		27.3061			
Treatments	23	178.942			
Factors 1	2	13.9914	6.9957	0.892948	.4161 NS
2	7	53.8575	7.69393	0.982072	0.4553 NS
Interactions 1 x 2	14	111.093	7.93519	1.01287	0.4568 NS
Error	48	376.051	7.83439		
Residual	0	.000427			

TABLE 3b

MOISTURE CONTENT OF SOYBEAN LEAVES AFTER EXPOSURE OF SO_2 GAS AT VARIOUS PERIODS OF TIME

A. O. V.

Source	d.f.	SS	MS	f	P
Total	72	37.1842			
Mean-1		36.6939	36.6939		
Treatments	23	0.481987			
Factors 1	2	0.250853	0.125426	772.818	P .01 S
2	7	.0903778	.012911	74.4053	P .01 S
Interactions 1 x 2	14	0.140748	0.0100534	57.9366	P .01 S
Error	48	0.00832915	.000173524		
Residual	0	.0000188			

TABLE 4

DRY WEIGHTS OF ONE GRAM EACH LEAF TISSUE AFTER SO_2 GAS FUMIGATION

Time (hrs)	SO_2 Gas ppm 0.2 gm*	0.2 Percent Change**	0.5 gm*	0.5 Percent Change**	0.8 gm*	0.8 Percent Change**
Lab (Cont)	0.25 ± 0.003		0.25 ± 0.003		0.26 ± 0.003	
Cham (Cont)	0.25 ± 0.003		0.25 ± 0.003		0.25 ± 0.003	
½	0.25 ± 0.003	0.0	0.23 ± 0.006	-8.0	0.31 ± 0.019	+ 19.2
1	0.24 ± 0.006	- 4.0	0.23 ± 0.006	-8.0	0.35 ± 0.012	+ 34.6
2	0.23 ± 0.009	- 8.0	0.24 ± 0.007	-4.0	0.37 ± 0.009	+ 42.3
4	0.22 ± 0.003	-12.0	0.24 ± 0.006	-4.0	0.39 ± 0.007	+ 50.0
6	0.25 ± 0.003	0.0	0.24 ± 0.003	+4.0	0.51 ± 0.006	+ 96.1
8	0.26 ± 0.006	+ 4.0	0.27 ± 0.003	+8.0	0.53 ± 0.012	+103.8

*The mean ± standard error, results of three individual experiments.

**Percent change calculated as $\frac{\text{Control - Experimental}}{\text{Control}} \times 100$. + ve and - ve values indicate increase and decrease of the content.

TABLE 5a

TOTAL PROTEIN CONTENT OF SOYBEAN LEAVES (mg/gm WET TISSUE)

A. O. V.

Source	d.f.	SS	MS	f	P
Total	72	17462.1			
Mean-1		14291.8	14291.8		
Treatments	23	3151.11			
Factors 1	2	1439.68	719.838	1795.37	P .01 S
2	7	721.398	103.057	257.038	P .01 S
Interactions 1 x 2	14	990.033	70.7166	176.377	P .01 S
Error	48	19.2452	0.400941		
Residual	0	0.00737			

TABLE 5b

SOLUBLE PROTEIN CONTENT OF SOYBEAN LEAVES (mg/gm of WET TISSUE)

A. O. V.

Source	d.f.	SS	MS	f	P
Total	72	13936.0			
Mean-1		11070.7	11070.7		
Treatments	23	2850.52			
Factors 1	2	1433.46	716.732	2337.26	P .01 S
2	7	551.68	78.8115	257.004	P .01 S
Interactions 1 x 2	14	865.376	61.8126	201.571	P .01 S
Error	48	14.7194	.306654		
Residual	0	.0008144			

TABLE 5c

INSOLUBLE PROTEIN CONTENT OF SOYBEAN LEAVES

A. O. V.

Source	d.f.	SS	MS	f	P
Total	72	168.1			
Mean-1		153.125	153.125		
Treatments	23	13.8084			
Factors 1	2	6.67583	3.23792	133.219	P .01 S
2	7	3.45722	0.493889	20.3203	P .01 S
Interactions 1 x 2	14	3.87528	0.276806	11.3887	P .01 S
Error	48	1.16665	0.024305		
Residual	0	.0000389			

TABLE 6

TOTAL PROTEIN CONTENT OF SOYBEAN LEAVES
(mg/gm Dry Tissue)

A. O. V.

Source	d.f.	SS	MS	f	P
Total	72	294339			
Mean-1		227981.0	227981.0		
Treatments	23	65537.6			
Factors 1	2	31978.1	15989.0	935.692	P .01 S
2	7	13420.8	1917.26	112.2	P .01 S
Interactions 1 x 2	14	20138.7	1438.48	84.1808	P .01 S
Error	48	820.221	17.0874		
Residual	0	0.04479			

TABLE 7

INSOLUBLE PROTEIN CONTENT SOYBEAN LEAVES AS AFFECTED BY SO_2 GAS EXPOSURE

ppm	Albumin 400 µg/ml	Control Lab.	O. D. X 10^{-3} at 500 mµ Control* Chamber	Hours Exposed ½	1	2	4	6	8
0.2	500	65	78	83	100	122	83	88	75
	470	72	71	88	86	98	96	86	83
	480	63	68	100	88	95	99	78	78
0.5	487	76	64	83	100	98	91	47	49
	485	71	70	93	88	88	87	54	34
	469	79	76	74	86	96	100	61	49
0.8	490	70	70	68	44	47	38	29	26
	495	80	65	59	49	42	32	37	23
	485	69	76	76	54	42	51	44	29

*Plants were exposed to air in the chamber, the data shown serially in the column are for 4, 6, and 8 hours.

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TABLE 8

TOTAL PROTEIN CONTENT OF THE SOYBEAN LEAVES AFTER RESPECTIVE RECOVERY PERIODS (SO_2 EXPOSURE)

A. O. V.

Source	d.f.	SS	MS	f	P
Total	63	17660.2			
Mean-1		17650.3	17650.3		
Treatments	20	3.29297			
Factors 1	2	1.84667	0.9233	5.9189	0.0054 S
2	6	0.79747	0.13291	0.8520	0.5377 NS
Interactions 1 x 2	14	0.651115	0.0542596	0.155997	
Error	42	6.55188	0.155997		
Residual	0	.007228607			